THE UNIVERSITY OF MANITOBA

STUDIES ON TETRATHYRIDIAL POPULATIONS OF *MESOCESTOIDES CORTI* HOEPPLI, 1925 (CESTODA: CYCLOPHYLLIDEA) IN MICE: EFFECTS OF STEROID HORMONES, CASTRATION AND SPLENECTOMY

by

MARIE NOVAK

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MARIE NOVAK

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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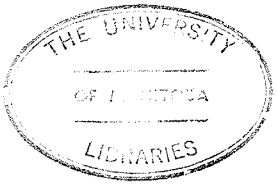

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I wish to express my deep gratitude to my supervisor Dr. G. Lubinsky for his suggestion to work with tetrathyridia of Mesocestoides - these fascinating splitting cestodes - and to investigate new aspects of host-parasite relationships, whose existance was suggested by results of the previous work with Echinococcus multilocularis.

I am also indebted to Dr. H.E. Welch, Head of Department of Zoology, for his kind permission to work in the Department, and to members of my committe for their constructive criticism of my work.

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Thanks are due to Mr. W. Heck for photographic work, and to my husband George for his patience and understanding during my studies.

ABSTRACT

The volumes of tetrathyridial populations in the peritoneal cavity of mice increase exponentially with time. Thirty days post infection more larvae are in this cavity than in the liver. After that the increase of intraperitoneal populations continues, whereas the infection of the livers remains more or less constant.

Splenectomy and treatment with testosterone, oestradiol, and cortisone increase the total volumes of intraperitoneal populations in hosts of both sexes. Their efficiency decreases in the following order: cortisone > testosterone > oestradiol = splenectomy > control.

Gonadectomy depresses considerably the volumes of intraperitoneal populations, orchiectomy being more efficient than ovariectomy. Testosterone increases considerably the size of tetrathyridial populations in castrates of both sexes, while oestradiol is much less efficient.

Both gonadectomy and the treatment with steroid hormones alter the infection of livers. Orchiectomy lowers it, whereas ovariectomy and steroid hormones increase it.

There exists a negative correlation between the size of populations of tetrathyridia and the average size of larvae: the larger the populations, the smaller the larvae. Thus the tetrathyridia from orchiectomized mice are the largest and those from cortisone treated males the smallest.

The sole factor found hitherto to depress the volumes of tetrathyridial populations is castration of the host.

INTRODUCTION

INTRODUCTION

The plerocercoid larvae, "tetrathyridia", of Mesocestoides corti Hoeppli, 1925, are the only known metacestodes multiplying by longitudinal fission. The intensity of their multiplication varies greatly from host to host. In some hosts, e.g. the muskrat, the multiplication is very slow, in others, as e.g. the jirds or the LDF_1 hybrid mice, very fast (Novak, 1972).

In this last study it was shown that the populations of tetrathyridia (Fig.1.) in contrast to the Echinococcus multilocularis cysts, grow much faster in male hosts, than in females. This suggested a study of the effect of sex hormones and of gonadectomy on the growth and the polymorphism of tetrathyridial populations in mice. Another steroid hormone, cortisone, suppresses the inflammatory reactions and the immunological responses in general, and is known to suppress the immunity to metacestodes (Olivier, 1962; Esch, 1964 & 1967; Lee, 1970). The present study was therefore extended to include three steroid hormones: testosterone, oestradiol, and cortisone (Fig.2.). As this last hormone depresses the reticuloendothelial system, it was interesting to compare its effect with that of splenectomy. Thus originated the plan of the present thesis, which comprises 4 chapters: 1. Effect of sex hormones on the growth and multiplication of tetrathyridia of M. corti in mice. 2. Gonadectomy, sex hormones, and the growth of tetrathyridial populations of M. corti in mice. 3. Cortisone and the growth of populations of Mesocestoides tetrathyridia in mice.

4. Acceleration of the growth of tetrathyridial populations of M. corti by splenectomy. In the course of the preliminary work on the effect of oestradiol on the tetrathyridial populations it was found that this hormone increases considerably the invasion of the liver. This observation was published as a letter to the editor in the Transactions of the Royal Society of Tropical Medicine and Hygiene. It appears as an introduction to the first part of this thesis.

Figure 1.

Population of tetrathyridia of *Mesocestoides corti* from the peritoneal cavity of an SEC mouse; x 4.

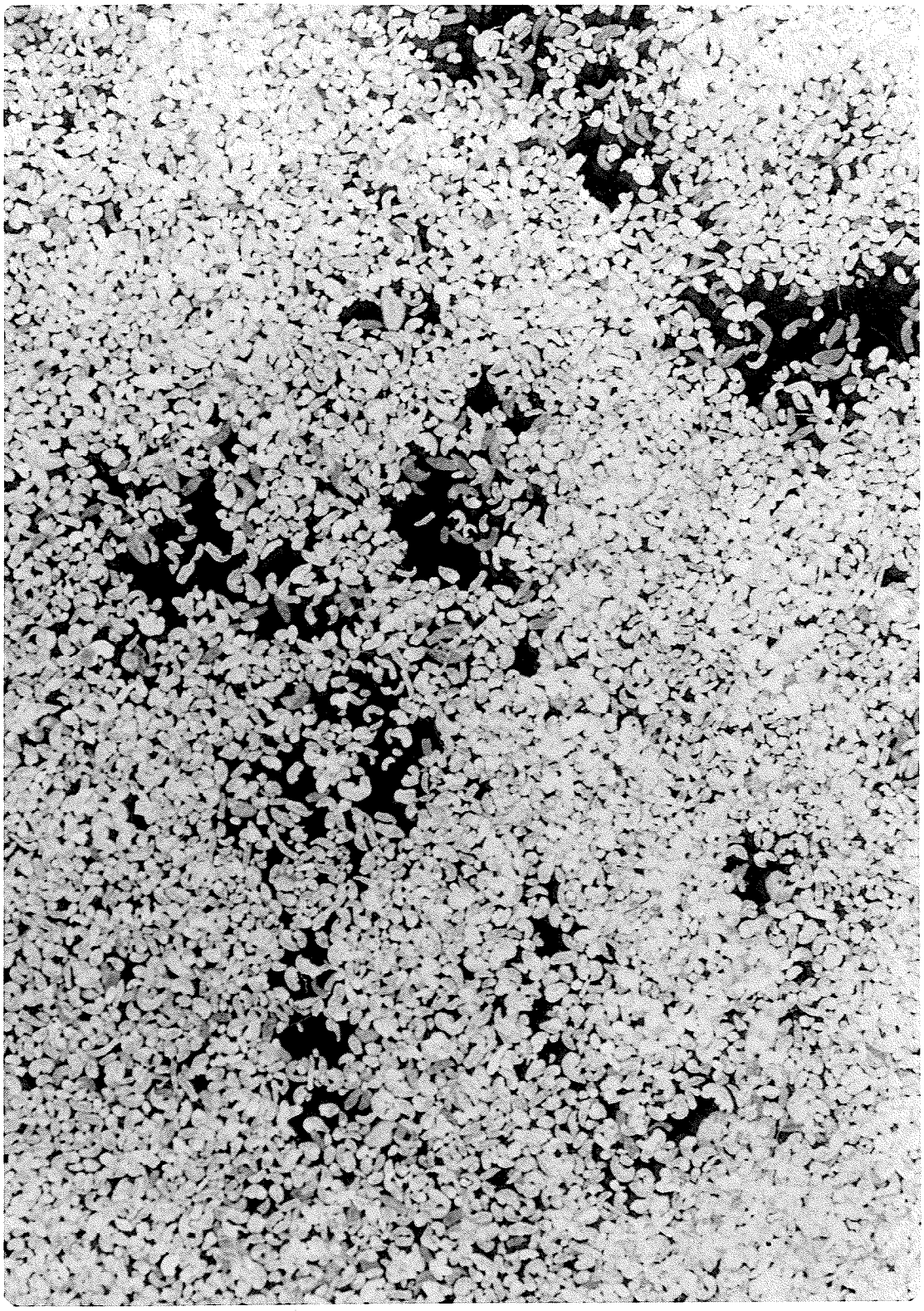


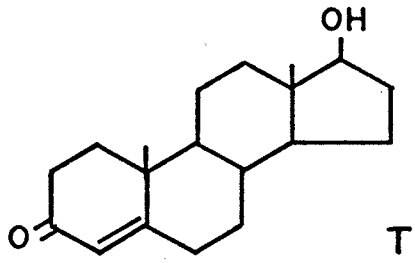
Figure 2.

Steroid hormones used in the present study:

testosterone

oestradiol

cortisone



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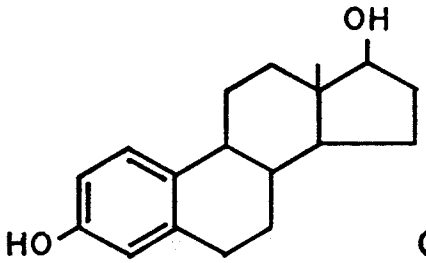
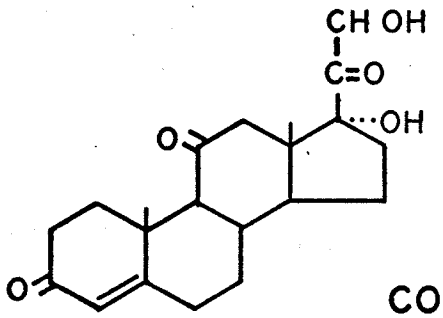


OESTRADIOL



CORTISONE

PART I.

EFFECT OF ESTRADIOL ON THE INVASION OF MICE LIVERS BY MESOCESTOIDES TETRATHYRIDIA.

Sir, - The fact that tetrathyridia of Mesocestoides corti grow and multiply faster in male hosts than in females (Novak 1972), led me to study the effects of sex hormones on the growth and multiplication of these proliferating plerocercoids. In this work an interesting observation was made on the effect of estradiol on the invasion of livers by tetrathyridia.

LDF_1 hybrid mice 6 months old were infected intraperitoneally with tetrathyridia. In the preliminary experiment 15 mice of each sex were used in each of the control, estradiol treated and testosterone treated groups. In the replication the number of mice per group was increased to 20.

Estradiol benzoate, 5 µg/g, and testosterone propionate, 10 µg/g, (Nutritional Biochemicals Corporation, Cleveland, Ohio) were injected subcutaneously twice a week and a total of ten injections given. At autopsy the volumes of tetrathyridial populations from the body cavities were measured. The livers, into which many plerocercoids penetrated, were weighed, and infection estimated macroscopically as light (1-10 larvae cm^{-2} of liver surface), moderate (10-50 larvae cm^{-2}) and heavy (more than 50 larvae

cm^{-2}). To determine the number of larvae in the livers these were digested with 1 per cent trypsin at pH 8.0 and 37°C.

In two experiments lasting 52 and 56 days, control females had less larvae than control males, the mean volumes of larval populations from peritoneal cavities being 0.34 ± 0.03 ml and 0.40 ± 0.02 ml in females and 0.36 ± 0.04 ml and 0.45 ± 0.02 ml in males. Testosterone increased the volume of larval populations from 0.34 ± 0.03 to 0.77 ± 0.05 ml and 0.40 ± 0.02 to 0.79 ± 0.03 ml in females, and from 0.36 ± 0.04 to 0.90 ± 0.05 ml and 0.45 ± 0.02 to 0.87 ± 0.03 ml in males. The P values were always < 0.001. Estradiol increased the mean volumes of intraperitoneal populations slightly, but consistently, in females from 0.34 ± 0.03 to 0.37 ± 0.04 ml and 0.40 ± 0.02 to 0.46 ± 0.01 ml, and in males from 0.36 ± 0.04 to 0.49 ± 0.03 ml and 0.45 ± 0.02 to 0.53 ± 0.03 ml. Yet it changed drastically the invasion of livers by tetrathyridia. In all mice receiving estradiol the invasion was graded semi-quantitatively as " High ", whereas that in the control females was " Low " and in control males " Moderate ". Liver infection of testosterone treated females was " Light-Moderate " and that of males " Moderate-Heavy ". The weights of livers of testosterone treated mice increased but slightly, but those in estradiol treated mice increased by 31 to 78 per cent.

Trypsin digestion of 10 livers taken at random from each of three groups of mice from experiment 2 showed that liver of control females harbored 358.40 ± 33.80 larvae, those of control males 835.90 ± 16.09 larvae. Testosterone slightly increased the number of larvae in the livers of both females (to 407.10 ± 24.16 larvae) and males (to 873.60 ± 16.36 larvae). In estradiol treated females the number of tetrathyridia in livers increased to 1173.00 ± ± 32.06, and in males to 1293.70 ± 48.95. It is evident that estradiol increased considerably the invasion of livers by tetrathyridia.

I am, etc.,

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19 March 1973.

REFERENCES

NOVAK,M. (1972). Can. J. Zool., 50, 1189.

APPENDIX

TABLE 1

Mean volumes of larval populations from peritoneal cavities of LDF_1 mice treated with testosterone and estradiol.

Exp.	Sex	Age Months	Group	Number of mice	Mean volumes of coelomic populations ml	P
1 56 days	M	6	Control	14	0.36 ± 0.04	-
			Testosterone	15	0.90 ± 0.05	< 0.001
			Estradiol	14	0.49 ± 0.03	< 0.05
	F		Control	15	0.34 ± 0.03	-
			Testosterone	15	0.77 ± 0.05	< 0.001
			Estradiol	12	0.37 ± 0.04	not sign.
2 52 days	M		Control	20	0.45 ± 0.02	-
			Testosterone	19	0.87 ± 0.03	< 0.001
			Estradiol	20	0.53 ± 0.03	< 0.05
	F		Control	20	0.40 ± 0.02	-
			Testosterone	20	0.79 ± 0.03	< 0.001
			Estradiol	19	0.46 ± 0.01	< 0.05

TABLE 2

The mean liver weights of controls, and of testosterone and estradiol treated LDF$_1$ mice.

Exp.*	Sex	Group	Liver weight g	Liver infection
1	M	Control	3.57 ± 0.13	M
		Testosterone	4.67 ± 0.13	M-H
		Estradiol	5.10 ± 0.17	H
	F	Control	2.68 ± 0.10	L
		Testosterone	3.11 ± 0.07	L-M
		Estradiol	4.77 ± 0.14	H
2	M	Control	3.27 ± 0.06	M
		Testosterone	3.28 ± 0.12	M-H
		Estradiol	4.28 ± 0.10	H
	F	Control	2.79 ± 0.09	L
		Testosterone	2.84 ± 0.08	L-M
		Estradiol	3.96 ± 0.08	H

* Duration of experiments and number of mice are the same as in Table 1.

TABLE 3

Mean number of larvae collected from livers of 10 LDF_1 mice of each sex from experiment 2.

Group	Males	Females
Control	835.90 ± 16.09	358.40 ± 33.80
Testosterone	873.60 ± 16.36	407.10 ± 24.16
Estradiol	1293.70 ± 48.95	1173.00 ± 32.06

EFFECT OF SEX HORMONES ON THE GROWTH AND MULTIPLICATION OF TETRATHYRIDIA OF *MESOCESTOIDES CORTI* (CESTODA: CYCLOPHYLLIDEA) IN MICE.

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Received.....1973

Mesocestoides tetrathyridia and sex hormones

Abstract

The number of tetrathyridia in the peritoneal cavities of mice increases exponentially with time. Thirty days post infection more larvae are in the cavities than in the livers. After that the increase of intraperitoneal populations continues, whereas the number of tetrathyridia in the livers remains more or less constant.

Exogenous testosterone propionate, 10 µg/g, twice a week, for 5 weeks, increases significantly the total volumes of tetrathyridial populations in the peritoneal cavities, whereas oestradiol benzoate, 5 µg/g or 10 µg/g, also for 5 weeks, accelerates the rate of growth and multiplication of coelomic tetrathyridia to a much lesser extent, but increases significantly the infection of the livers.

INDEX KEY WORDS: *Mesocestoides corti*, tetrathyridia, larval populations, LDF_1 hybrid mice, SEC mice, testosterone propionate, oestradiol benzoate.

INTRODUCTION

AN IMPORTANT factor in the incidence and severity of parasitic diseases is the sex of the host and it depends, at least to some extent, on the sex hormones. Solomon (1969) reviewed the literature on the hormonal influence of the host on infections with "microorganisms, protozoa, and metazoa".

The influence of the host's sex and of sex hormones on infections with larval cestodes was studied by Baron (1971), Culbreth et al. (1972), Frayha et al. (1971), Freeman (1962) and Lubinsky (1967).

Recent studies (Novak, 1972; Novak & Lubinsky, 1973) showed that populations of tetrathyridia of *Mesocestoides corti* grow faster in male than in female mice. This suggested a study of the influence of sex hormones on the growth and multiplication of these metacestodes.

MATERIALS AND METHODS

LDF_1 mice (first generation hybrids between C57L females and DBA_1 males) and those of SEC strain were infected intraperitoneally with 0.03 ml of sedimented larvae of *Mesocestoides corti*. All mice were 4 - 6 months old. The age of mice used in each experiment is given in the tables. Tetrathyridia were kindly donated in 1968 by Professor M. Voge,

U.C.L.A., and the strain maintained in our laboratory in LDF_1 mice which were free of other helminths. Mice were kept five per cage at 20°C and 50% humidity, and fed Purina Lab chow and water ad libitum. The light cycle was 15 h stimulated daylight and 9 h darkness.

Treatment with sex hormones was started one day after infection. Mice were divided into controls, testosterone, and oestradiol treated groups. Testosterone propionate, 10 µg/g, and 17-β oestradiol benzoate, 5 or 10 µg/g (Nutritional Biochemicals Corporation, Cleveland, Ohio) were given subcutaneously as a suspension in corn oil. Injections were made twice a week with alternating 3 and 4 days invervals. In all experiments a total of 10 injections was given. In the present paper expressions "5 µg/g" and 10 µg/g" will be used to mean 10 injections of these doses.

Twenty LDF_1 males and twenty females received a total of ten injections of 0.03 ml of corn oil. This oil alone had no effect on the growth of tetrathyridial populations.

Mice were autopsied 50 - 56 days post infection. Tetrathyridia were washed from peritoneal cavities with balanced salt solution, each population placed in a vertically standing tuberculin syringe and left to sediment for 10 minutes. The volumes were recorded with an exactitude of 0.01 ml. Livers were weighed and the intensity of infection estimated macroscopically as light, moderate or heavy (Novak, 1972). Livers of some mice were cut

into small pieces and digested with 1% trypsin solution at pH 8.0 and 37°C for 8 - 12 hours, and larvae counted. As single larvae were regarded all stages of longitudinal splitting up to complete separation, independently of the number of scoleces. Acephalic fragments were also included in this count. Polycephalic forms - those with 3 or more scoleces - occur only in populations older than 3 months and were not encountered in the course of the present work.

The tetrathyridia are solid plerocercoid-type larvae without bladders. When degenerating, they seldom vacuolate, and the volume of sedimented tetrathyridial populations seems to be a reasonably good measure of the biomass of these populations.

Data were analyzed using Student's t-test. Differences were accepted as significant when $P<0.05$.

RESULTS

In the first experiment, involving 25 male LDF_1 mice, changes in the volumes of populations in the peritoneal cavities and in the livers were studied. Five mice were dissected every 10 days, 10 - 50 days post infection, and the larvae counted (Table 1). Only at one point, twenty days post infection, did the number of larvae in the livers exceed that in the peritoneal cavities, at all other times the reverse was true. Numbers of

larvae in the livers attained their maximum 30 days post infection, and levelled off at about 900 - 1000 larvae. Weight of the livers increased even after this maximum was reached, and was 50 days post infection more than double that in mice autopsied 10 days post infection, 3.43 and 1.60 g respectively. The average weight of livers of uninfected control males of the same age, i.e. 4 months plus 50 days, was 1.52 $\pm$ 0.03 grams, thus smaller than that of livers of infected males which were 40 days (50 minus 10 days) younger. As the volume of 1000 larvae is about 0.125 ml, it is obvious that the doubling of the weights of livers 40 and 50 days post infection depends mainly on the inflammation caused by the tetrathyridia, and not on the weight of the invading parasites.

The volume of intraperitoneal tetrathyridial populations increased rapidly and at the end of the experiment almost four times more tetrathyridia were in the peritoneal cavity than in the liver. The growth of the volumes of peritoneal populations seems to be exponential and is not inhibited at least within the first 50 days post infection.

Figure 1 (experiments 2 - 5) shows the influence of testosterone and of oestradiol on the growth of intraperitoneal tetrathyridial population in LDF_1 and SEC mice of both sexes. Testosterone propionate, 10 µg/g, increased the volume of larval populations 82% in SEC males in experiment 5, and 150% in LDF_1

males in experiment 2. The increase in the volume of tetrathyridial populations in females ranged from 108% in SEC mice in experiment 5 to 126% in LDF_1 in experiment 2. All differences were statistically significant ($P<0.001$).

The effect of oestradiol benzoate, 5 µg/g, was much less pronounced and ranged from a 6% increase in volume of larval populations in SEC males in experiment 5 to 36% in LDF_1 males in experiment 2, and from 9% in LDF_1 females in experiment 2 to 30% in SEC females in experiment 4. Though differences were not statistically significant, they occurred in all experiments, and were probably real.

In experiments 2 - 5 the dose of oestradiol, 5 µg/g, was half the dose of testosterone, 10 µg/g, the dosage used by Baron (1971) with *E. multilocularis*. It was important to determine whether a double dose of oestradiol, 10 µg/g, would further accelerate the growth of intraperitoneal larval populations and the invasion and enlargement of the livers. Two experiments, 6 and 7, (Table 2) showed, that this dose given ten times, did not produce a statistically significant increase in the volumes of intraperitoneal populations over those observed in mice treated with the smaller dose. However, the livers of all mice treated with oestradiol, either 5 or 10 µg/g, were heavily infected and their average weights significantly higher ($P<0.001$) than those of livers of infected

but not treated controls. The livers of females receiving 10 µg/g of oestradiol weighed 5 - 9% more than those of females treated with 5 µg/g, but the difference was significant only in experiment 7. There was no difference in the weight of livers of males treated with two different doses of oestradiol.

The invasion of the liver and the increase in its weight were studied in more detail in mice from experiments 2 - 5. Increase in the liver weights of LDF_1 and SEC mice (4 - 6 months old at infection) given testosterone was none to 31% in males and 2 - 15% in females, that in oestradiol treated mice 31 - 53% in males and 42 - 95% in females in comparison to the liver weights of infected but not treated controls. Associated with this increase in liver weights was an increase in the number of tetrathyridia in the livers. Testosterone, 10 µg/g, increased the number of tetrathyridia in the livers of mice of both sexes but slightly, from "light" to "light-moderate" in females, and from "moderate" to "moderate-heavy" in males. Livers of mice of both sexes treated with oestradiol, 5 µg/g, were heavily infected.

To study the intensity of invasion quantitatively, livers of 10 LDF_1 mice of each sex taken at random from the second experiment were digested with trypsin and the larvae counted (Table 3). Differences in the number of larvae between controls and testosterone treated animals were not significant. Oestradiol, 5 µg/g, increased the number of tetrathyridia in the livers by about 55% in males, and by over 200% in females. As 1 ml of

sedimented tetrathyridia contains about 8000 larvae, the total volume of tetrathyridia in the livers of oestradiol treated mice was about 0.17 ml, whereas the volumes of populations from the livers of testosterone treated and control mice varied from 0.05 - 0.11 ml. Thus the total volumes of the populations (from the peritoneal cavities plus those from the livers) of oestradiol treated mice were still smaller than those from testosterone treated animals.

Histological examination of the livers of oestradiol treated mice revealed a heavy infiltration by mononuclears—lymphocytes, monocytes and especially plasma cells. Pronounced hepatomegaly of oestradiol treated mice was always correlated with considerable splenomegaly.

DISCUSSION

The present experiments showed that 30 days post infection more tetrathyridia are in the peritoneal cavities of the mice than in their livers. Whereas liver infection reaches its maximum at this time, the increase in the total volumes of intraperitoneal populations continues.

Both testosterone and oestradiol accelerate the multiplication of tetrathyridia, but whereas testosterone increases the volume of their intraperitoneal populations,

oestradiol causes an increase in the number of tetrathyridia present in the liver.

A decrease of the resistance of rats injected testosterone to experimental infections with the strobilocerci of *Hydatigera taeniaeformis* was observed by Campbell as early as in 1939. Frayha, Lawlor, and Dajani (1971) showed that testosterone increases both the number and the size of the cysts of *Echinococcus granulosus* in experimental mice of both sexes.

My experiments showed that oestradiol increases the volumes of intraperitoneal tetrathyridial populations of *Mesocestoides corti* to a lesser extent than testosterone, even when given in the same dose. This contrasts with the findings of Baron (1971) who, working with *Echinococcus multilocularis*, stated that "oestradiol in a dose half that of testosterone accelerated the growth of cysts as much as testosterone did". This difference may be related to the fact that *E. multilocularis* cysts grow faster in female mice (Lubinsky, 1967), whereas the population of *Mesocestoides* tetrathyridia increase in their volume faster in male hosts than in females (Novak, 1972).

Acknowledgements - This research was supported by the National Research Council of Canada Grant A - 1426 to Professor G. Lubinsky.

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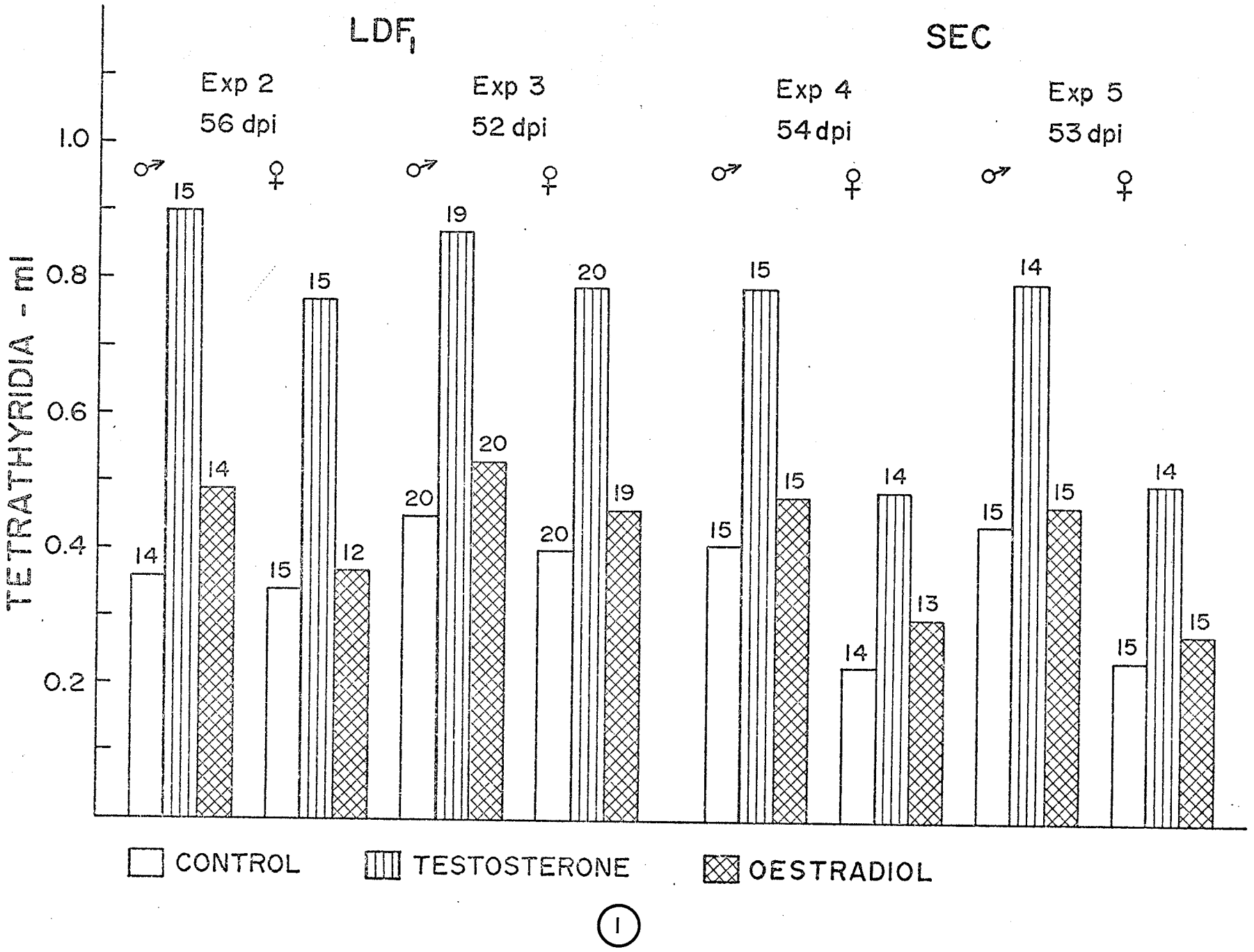
(14)

Figure 1.

Mean volumes of tetrathyridial populations in control, testosterone, and oestradiol treated LDF_1 and SEC mice from experiments 2 to 5. The numbers above the bars represent the number of animals used.


LDF1
SEC
Exp 2
56 dpi
Exp 3
52 dpi
Exp 4
54 dpi
Exp 5
53 dpi
♂
♀
TETRATHYRIDIA - ml
1.0
0.8
0.6
0.4
0.2
15
14
15
12
19
20
20
19
15
15
14
13
14
15
15
14
CONTROL
TESTOSTERONE
OESTRADIOL

(1)

TABLE 1.

NUMBERS (mean ± S.E.) OF LARVAE IN LIVERS AND PERITONEAL CAVITIES OF 5 GROUPS OF LDF_1 MALE MICE, 4 MONTHS OLD, AND DISSECTED 10-50 DAYS POST INFECTION (5 mice / sample).

Days post infection	Liver		Peritoneal cavity	
	Weight g	Numbers of larvae	Volume of populations ml	Numbers of larvae
10	1.60 ± 0.08	162 ± 26	0.04 ± 0.00	384 ± 51
20	2.42 ± 0.07	869 ± 33	0.07 ± 0.00	586 ± 68
30	2.73 ± 0.14	1107 ± 69	0.21 ± 0.04	1676 ± 281
40	3.01 ± 0.11	929 ± 120	0.27 ± 0.03	2178 ± 242
50	3.43 ± 0.19	936 ± 115	0.56 ± 0.05	4451 ± 402

TABLE 2.

VOLUMES OF INTRAPERITONEAL POPULATIONS AND LIVER WEIGHTS OF LDF_1 MICE, 6 MONTHS OLD, TREATED WITH OESTRADIOL, 5 OR 10 µg/g, AND DISSECTED 50 DAYS POST INFECTION.

Group	Sex	No. of mice	Volume of intrap. populations ml mean + S.E.	t	Liver weight g mean + S.E.	t	Liver infection
Exp. 6							
Control	M	20	0.38 ± 0.02		2.89 ± 0.06		Moderate
Oestradiol, 5ug/g	M	18	0.41 ± 0.01	1.3300	4.13 ± 0.05	0.7773	Heavy
Oestradiol,10 ug/g	M	18	0.39 ± 0.01		4.20 ± 0.08		Heavy
Control	F	20	0.32 ± 0.02		2.28 ± 0.05		Light
Oestradiol, 5ug/g	F	19	0.34 ± 0.01	1.1600	3.96 ± 0.06	1.5933	Heavy
Oestradiol,10 ug/g	F	18	0.33 ± 0.01		4.15 ± 0.10		Heavy
Exp. 7							
Control	M	20	0.55 ± 0.01		2.81 ± 0.09		Moderate
Oestradiol, 5ug/g	M	18	0.57 ± 0.01	1.7624	4.35 ± 0.07	0.7108	Heavy
Oestradiol,10 ug/g	M	20	0.62 ± 0.03		4.28 ± 0.08		Heavy
Control	F	15	0.44 ± 0.02		2.54 ± 0.08		Light
Oestradiol, 5ug/g	F	13	0.48 ± 0.03	0.1040	3.95 ± 0.10	2.1550	Heavy
Oestradiol,10 ug/g	F	18	0.48 ± 0.02		4.32 ± 0.14		Heavy

TABLE 3.

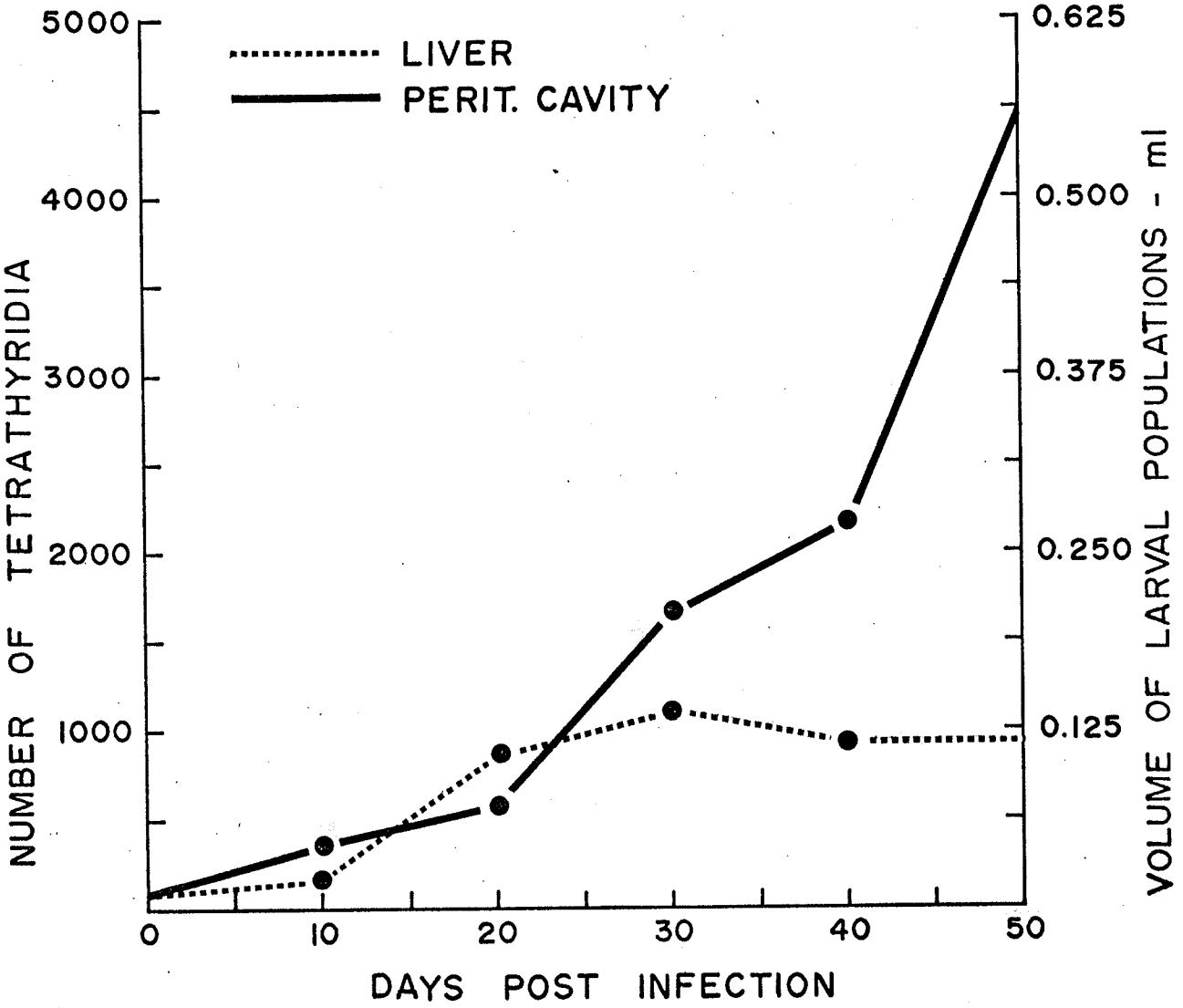
NUMBERS OF LARVAE (mean ± S.E.) IN THE LIVERS OF GROUPS OF 10 MICE TREATED WITH TESTOSTERONE OR OESTRADIOL (FROM EXPERIMENT 2).

Group	Numbers of larvae in males	Numbers of larvae in females
Control	836 ± 16	358 ± 34
Testosterone	874 ± 16	407 ± 24
Oestradiol	1294 ± 49	1173 ± 32

APPENDIX

Figure 2

Numbers of tetrathyridia and volumes of larval populations in livers and peritoneal cavities of 5 groups of LDF_1 male mice, 4 months old, dissected 10-50 days post infection ((5mice/sample).


LIVER
PERIT. CAVITY
NUMBER OF TETRATHYRIDIA
VOLUME OF LARVAL POPULATIONS - ml
5000
4000
3000
2000
1000
0.625
0.500
0.375
0.250
0.125
0
10
20
30
40
50
DAYS POST INFECTION

(2)

Figure 3.

The size (as area) and the number of suckers of 200 tetrathyridia from populations of a control, a oestradiol, and a testosterone treated SEC female from experiment 2.

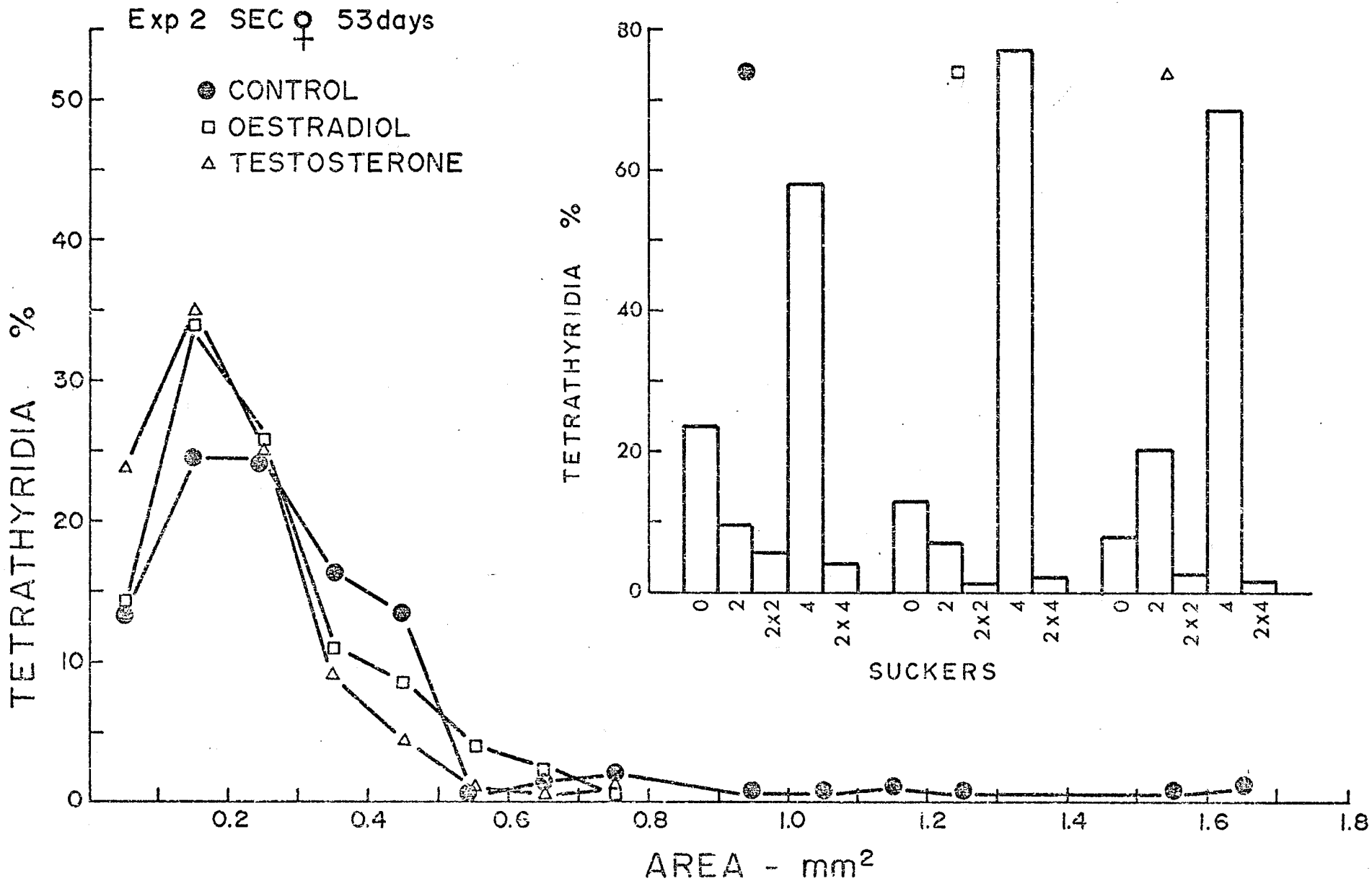

Exp 2 SEC ♀ 53days
CONTROL
OESTRADIOL
TESTOSTERONE
TETRATHYRIDIA %
AREA - mm²
0
10
20
30
40
50
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
TETRATHYRIDIA %
0
20
40
60
80
0
2
2x2
4
2x4
SUCKERS

Figure 4.

The size (as area) and the number of suckers of 200 tetrathyridia from populations of a control, a oestradiol, and a testosterone treated SEC male from experiment 2.

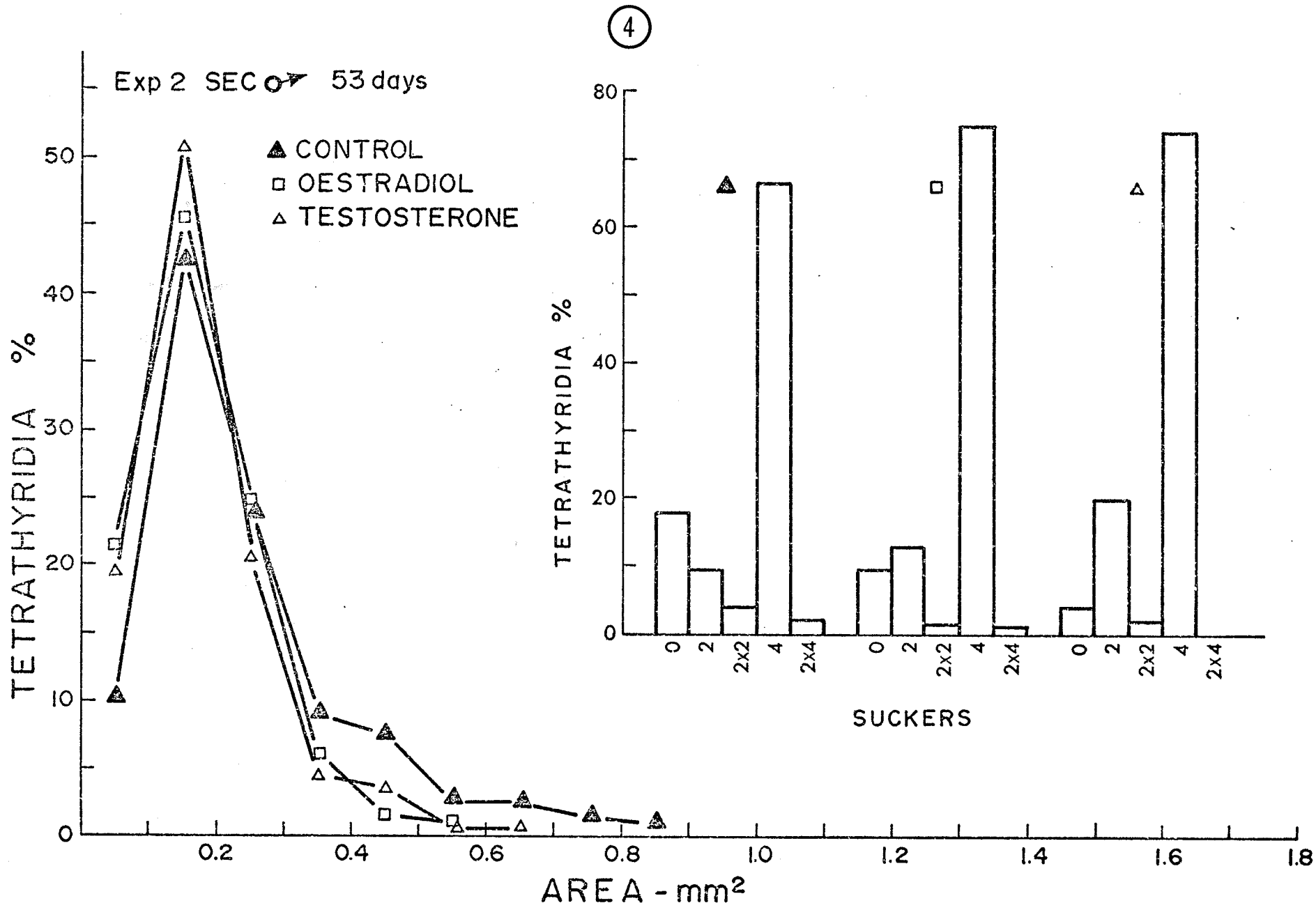

4
Exp 2 SEC ♂ 53 days
CONTROL
OESTRADIOL
TESTOSTERONE
TETRATHYRIDIA %
AREA - mm²
0
10
20
30
40
50
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
TETRATHYRIDIA %
0
20
40
60
80
0
2
2x2
4
2x4
SUCKERS

TABLE 4.

CONTROL AND CORN OIL INJECTED LDF_1 MICE INFECTED WITH TETRATHYRIDIA OF M. CORTI, AND DISSECTED 50 DAYS POST INFECTION.

Group	Sex	No. of mice	Larval popul. ml
Control	M	20	0.39 ± 0.01
Corn oil	M	20	0.41 ± 0.01
Control	F	20	0.34 ± 0.01
Corn oil	F	20	0.36 ± 0.01

TABLE 5.

LIVER WEIGHTS ($\bar{X} \pm \sigma_x$) OF CONTROL, TESTOSTERONE AND OESTRADIOL TREATED LDF_1 AND SEC MICE FROM EXPERIMENTS 2 to 5.

Group	Sex	Age at inf. months	No.of mice	Liver weight g	Liver infection
Exp.2, LDF_1 mice 56 days					
Control	M	6	14	3.57±0.13	Moderate
Testosterone			15	4.67±0.13	Moderate-Heavy
Oestradiol			14	5.10±0.17	Heavy
Control	F	6	15	2.68±0.10	Light
Testosterone			15	3.11±0.07	Light-Moderate
Oestradiol			12	4.77±0.14	Heavy
Exp.3, LDF_1 mice 52 days					
Control	M	4	20	3.27±0.06	Moderate
Testosterone			19	3.28±0.12	Moderate-Heavy
Oestradiol			20	4.28±0.10	Heavy
Control	F	4	20	2.79±0.09	Light
Testosterone			20	2.84±0.08	Light-Moderate
Oestradiol			19	3.96±0.08	Heavy
Exp.4, SEC mice 54 days					
Controls	M	4	15	2.56±0.08	Moderate
Testosterone			15	2.62±0.10	Moderate-Heavy
Oestradiol			15	3.57±0.10	Heavy
Controls	F	4	14	2.16±0.07	Light
Testosterone			14	2.36±0.07	Light-Moderate
Oestradiol			13	3.09±0.10	Heavy
Exp.5, SEC mice 53 days					
Controls	M	4	15	2.43±0.04	Moderate
Testosterone			14	2.65±0.09	Moderate-Heavy
Oestradiol			15	3.72±0.06	Heavy
Control	F	4	15	2.00±0.03	Light
Testosterone			14	2.30±0.06	Light-Moderate
Oestradiol			15	3.90±0.06	Heavy

PART II.

GONADECTOMY, SEX HORMONES, AND THE GROWTH OF TETRATHYRIDIAL POPULATIONS OF *MESOCESTOIDES CORTI* (CESTODA : CYCLOPHYLLIDEA) IN MICE

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Received.....1974

ABSTRACT

Gonadectomy in mice considerably depressed the volumes of intraperitoneal tetrathyridial populations, orchiectomy being more efficient than ovariectomy. Testosterone propionate accelerated the growth of tetrathyridial populations in castrates of both sexes. Oestradiol benzoate was less efficient, but considerably increased the invasion of livers both in intact and in gonadectomized mice. The average size of tetrathyridia was inversely proportional to the size of the populations: tetrathyridia from a castrated male were the largest, those from a castrated and testosterone-treated male, the smallest, and those from castrated male treated with oestradiol, intermediate in size.

INDEX KEY WORDS: *Mesocestoides*; tetrathyridia; larval populations; gonadectomy; testosterone; oestradiol.

INTRODUCTION

IN a previous paper Novak (1974b) showed that testosterone propionate and to a lesser extent oestradiol benzoate accelerate the growth of tetrathyridial populations of *Mesocestoides corti* in intact mice of both sexes. The aim of this paper is to study the influence of these hormones on the growth of populations and the morphology of tetrathyridia in gonadectomized mice.

MATERIALS AND METHODS

LDF_1 hybrids (C57L females x DBA_1 males) and SEC mice of both sexes, 3-8 months old, were used in the present study. They were gonadectomized a month before infection to allow for recovery and elimination of sex hormones. Mice were anaesthetized with ether and injected 1% aqueous solution of Avertin (tribromoethanol, Winthrop) prewarmed to 40°C, 0.1 mg/g of body weight. Narcosis lasted for 30-60 min. Testes with epididymes were removed through a 0.5-1.0 cm incision anteriorly to the pubis. In females, smaller incisions were made on both sides of the vertebral column, and the ovaries removed. The musculature was sutured with surgical silk Ethicon, 000, using a round-bodied #18 needle. Skin incisions

were clamped with 9 mm wound clips (Autoclips, Clay-Adams).

As in the previous work (Novak, 1974b) the treatment with sex hormones was started one day after infection. Testosterone propionate, 10 µg/g, and 17- β oestradiol benzoate, 5 µg/g (Nutritional Biochemicals Corporation, Cleveland, Ohio) were given subcutaneously as a suspension in corn oil. Injections were made twice a week in alternating 3 and 4 day intervals, and a total of 10 injections given. An increase in the dosage of oestradiol from 5 to 10 µg/g, did not change the results (Novak, 1974b) and only a lower dose was used in the present series of experiments.

In all experiments mice were divided into 4 groups: controls, castrated, castrated and testosterone treated, and castrated and oestradiol treated animals. They were dissected 50 days post infection.

Three hundred tetrathyridia were taken at random from populations from (1) one intact LDF_1 control male, (2) one castrated male, (3) one castrated and testosterone treated male, and (4) one castrated and oestradiol treated male. Larvae were allowed to relax in water and fixed in 10% formalin. Their body surfaces were measured with a planimeter, and the number of suckers recorded.

The techniques of infecting mice with tetrathyridia, of hormone treatment, and of the study of tetrathyridial populations were described previously (Novak, 1974a and b).

Student's t-test was used for statistical analysis. Probability levels greater than 5% were regarded as statistically not significant.

RESULTS

Orchiectomy decreased the volumes of intraperitoneal populations by 72 and 78% in LDF_1 mice in experiments 1 and 2 respectively, and by 60 and 71% in SEC mice in experiments 3 and 4 (Fig. 1). All differences were significant at $P < 0.001$.

The intensity of invasion of livers was roughly proportional to the volumes of intraperitoneal populations, and these were always smaller in castrates than in controls (Table 1). Livers of control males were "moderately" infected and had in the LDF_1 mice of experiments 1 and 2 average weights of 3.73 and 2.91 g, and in SEC mice of experiments 3 and 4, 2.75 and 2.74 g, respectively. Livers of orchiectomized LDF_1 mice were "very lightly" infected and weighed 2.72 and 2.93 g, those of orchiectomized SEC mice, 2.57 and 2.45 g, respectively.

Larvae from an intact LDF_1 male had a mean area of 0.29 ± 0.01 mm^2 with variation from 0.05 to 1.15 mm^2, and those from a castrate, 0.61 ± 0.02 mm^2 with variation from 0.05 to 2.15 mm^2, (Fig. 2).

The percentage of splitting larvae with two scolices with 4 suckers each ("2x4") was 1% in the intact male and 1.7% in the castrate. The prematurely splitting larvae, those with only 2 suckers per scolex ("2x2") were more frequent in the intact male than in the castrate, 1.3 and 0.3% respectively, and so were non-splitting larvae with 2 suckers, 16.7 and 9.0%, respectively, with $P < 0.01$. The number of acephalic forms was twice as high in the castrate as in the intact male, 19.3 and 0.7%, respectively, with $P < 0.001$. The decrease in the number of the immature tetrathyridia with 2 suckers, and the increase in that of acephalic forms in castrated males are probably the result of a slow-down in the multiplication of larvae.

Ovariectomy was less efficient in reducing the size of intraperitoneal populations than orchiectomy. A decrease in the volumes of these populations caused by ovariectomy was observed only in experiments 2 and 4, but not in 1 and 3. In experiment 2, that with LDF_1 mice, the volumes of populations from intact and ovariectomized mice were 0.53 and 0.45 ml, respectively, with $P < 0.001$,

and in experiment 4, with SEC mice, the corresponding figures were 0.38 and 0.21 ml, also with $P < 0.001$. There were no significant differences between these volumes in experiments 1 and 3.

Livers of control females were "lightly" infected and weighed on the average in LDF_1 mice from experiment 1 and 2, 3.03 and 2.31 g, and in SEC mice from experiment 3 and 4, 2.46 and 2.42 g, respectively. Livers of ovariectomized mice were infected "light-moderate" and weighed in LDF_1 mice 3.25 and 2.61 g, and in SEC mice, 2.75 and 2.53 g, respectively (Table I). Thus ovariectomy increased the weight of livers and the intensity of their infection.

Testosterone and the size of populations. Castrated LDF_1 males treated with testosterone had about 5 times larger populations, and the treated SEC castrates about 4 times larger than untreated castrates. The volumes of populations from testosterone treated castrates were on the average 40 and 90% larger in LDF_1 and SEC mice respectively, than those from intact males.

In all experiments with orchiectomized mice testosterone increased the infection of livers from "very light", in untreated to "moderate-heavy" in treated mice (Table I). Livers of treated castrates in experiment 1 and 2 weighed on the average 2.86 and 2.95 g, those from untreated castrates, 2.72 and 2.93 g, respectively.

Livers of SEC treated orchiectomized mice in experiment 3 and 4 weighed 2.66 and 2.95 g, those of untreated castrates, 2.57 and 2.45 g, respectively. These differences were not statistically significant, except in experiment 4.

Ovariectomized mice treated with testosterone had smaller intraperitoneal populations than the orchiectomized and testosterone treated mice in all but experiment 3, and the differences were statistically significant ($P < 0.001$).

Infection of livers in ovariectomized and testosterone treated mice increased in comparison with untreated castrates from "light-moderate" to "moderate". Livers of ovariectomized and treated LDF_1 mce from experiment 1 and 2 had average weights of 2.09 and 2.48 g, those of untreated castrates, 3.25 and 2.61 g, respectively. Livers of SEC treated ovariectomized mice in experiment 3 and 4 weighed 2.68 and 2.93 g, those of untreated castrates, 2.75 and 2.53 g, respectively.

Testosterone and the morphology of larvae.

Larvae from a castrated and testosterone treated LDF_1 male were larger than those from an intact control (0.34 ± 0.01 mm^2 and 0.29 ± 0.01 mm^2, respectively), but smaller than those from an untreated castrate (0.61 ± 0.02 mm^2). The distribution of larvae among the size and morphology groups in the population from the castrated and testosterone

treated male was almost identical to that from an intact male (Fig. 2). The number of larvae with 2 suckers was significantly higher ($P < 0.01$) in the treated male than in the untreated castrate males.

Oestradiol and the size of populations.

The mean volumes of intraperitoneal populations from orchiectomized mice treated with oestradiol were close to those of controls in experiments 1 and 3, but lower than that ($P < 0.001$) in experiments 2 and 4 (Fig. 1).

Infection of livers in oestradiol treated orchiectomized mice increased in comparison with treated castrates from "very light" to "heavy" (Table 1). The livers of treated orchiectomized LDF_1 mice from experiment 1 and 2 had on the average weights of 4.39 and 4.49 g, those of untreated castrates, 2.72 and 2.93 g, respectively. Livers of SEC treated orchiectomized mice in experiment 3 and 4 weighed 3.13 and 3.09 g, those of untreated castrates, 2.57 and 2.45 g, respectively.

Oestradiol increased the volumes of tetrathyridial populations in ovariectomized SEC mice, bringing them closer to or above those of intact females (Fig. 1). This was not observed in LDF_1 mice.

Oestradiol produced extremely heavy infections of the liver in ovariectomized mice. Their livers were significantly heavier than those of untreated ovariectomized females, which were only "light-moderate" infected (Table I). The livers of oestradiol treated LDF_1 castrates from experiment 1 and 2 weighed on the average 4.09 and 4.36 g, those of untreated castrates, 3.25 and 2.61 g, respectively. Livers of SEC treated ovariectomized mice in experiment 3 and 4 weighed 2.98 and 3.09 g, those of untreated castrates, 2.75 and 2.53 g, respectively.

Oestradiol and the morphology of larvae. The composition of the tetrathyridial population from a castrated and oestradiol treated male (Fig. 2) resembled that from a castrated and untreated male, though tetrathyridia from the treated mouse were smaller than those from the untreated castrate, 0.50 ± 0.02 and 0.61 ± 0.02 mm^2, respectively.

DISCUSSION

The present research showed that castration of male LDF_1 mice decreased the volumes of intraperitoneal populations of tetrathyridia by over 70%, and that of SEC males, by 60% or more. Ovariectomy reduced the size of these populations in only two out of the 4 experiments.

This reduction was 15% in LDF_1 mice in experiment 2, and 47% in SEC mice in experiment 4. Similar effects of gonadectomy on the growth of cysts of *Echinococcus multilocularis* in LDF_1 mice of both sexes were described by Baron (1971).

As in earlier experiments (Novak, 1974a; Novak and Lubinsky, 1973), the increase in the size of populations was negatively correlated with the average size of individuals: the larger the population, the smaller the individuals. This phenomenon was well expressed in LDF_1 males of the first two experiments, in which larvae from an intact male (with larger population) had an average area of 0.29 mm^2, larvae from a castrated male (castrates had smaller populations), an average area of 0.61 mm^2. Similar relationships were observed in other experiments of the present and earlier work.

50-60 days post infection the intraperitoneal populations of tetrathyridia in mice are relatively small, and the decrease in the size of individuals can hardly be explained as result of crowding. In fast growing populations of this age, the increase in the percentage of dividing larvae with only 2 suckers per scolex suggests that the decrease in the average size of individuals is the result of accelerated multiplication, and not of crowding.

In the present series of experiments the most efficient means of accelerating the growth of tetrathyridial populations was testosterone. In experiment 2 with LDF_1 mice, the average volume of populations from castrated testosterone treated males was about 0.9 cc, almost six times larger than that from castrated but untreated males (Fig. 1).

It is remarkable that both testosterone and oestradiol increased the size of tetrathyridial populations in castrated males more than in castrated females, despite an interval of 30 days between castration and infection, a phenomenon worthy of further investigation.

Both these hormones alter the effect of castration on infection of rodents with larval *Taenia taeniaeformis* and *E. multilocularis*. Testosterone lowers the resistance of ovariectomized rats to the strobilocerci of *T. taeniaeformis* whereas oestradiol increases the resistance of castrated males to these cestodes (Campbell and Melcher, 1940). Both testosterone and oestradiol accelerate the growth of cysts of *E. multilocularis* in castrated LDF_1 mice of both sexes (Baron, 1971).

The finding that gonadectomy depresses the liver infection in males, but enhances it in females, was unexpected. Whereas the "very low" infections of livers

in castrated males as compared to "moderate" in intact males can be explained as a suppression of the growth of populations by castration, the "light-moderate" infections of livers in ovariectomized mice as compared to "light" infections in intact females are not easily understood. Indeed, sham operations do not affect the growth of tetrathyridial populations (Novak, 1974a), and the removal of the ovaries - the main source of oestrogens, could be expected to depress the invasion of the liver. Yet the intensification of the liver invasion in ovariectomized mice was observed in all four experiments and is almost certainly real. A similar observation was made by Campbell and Melcher (1940), who found more strobilocerci of *T. taeniaeformis* in livers of ovariectomized rats than in controls. However, the strobilocerci develop from oncospheres, and they do not multiply asexually.

Testosterone increased the infection of the livers from "very light" in nontreated castrated males to "moderate-heavy" in treated ones. In females, it increased the infection from "light-moderate" in untreated castrates, to "moderate" in treated ones. It was thus less efficient in castrated females than in males.

As in the previous experiments with noncastrated mice, (Novak, 1973; 1974b), oestradiol enhanced liver infections in both sexes of mice. All oestradiol treated animals had "heavy" infections of the liver.

Present experiments showed that the most efficient means of increasing the tetrathyridial populations in mice was testosterone treatment, and the most efficient means of inhibiting the growth of these populations was the castration of males. Further research may show whether other steroid hormones e.g. corticosteroids, may be as efficient as testosterone in increasing populations of larval *Mesocestoides*.

ACKNOWLEDGMENTS

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Figure 1.

Mean volumes of tetrathyridial populations in control, castrated, castrated and testosterone treated, and castrated and oestradiol treated LDF_1 and SEC mice, from experiments 1 to 4, 50 days post infection. The numbers above the bars represent the number of animals used.

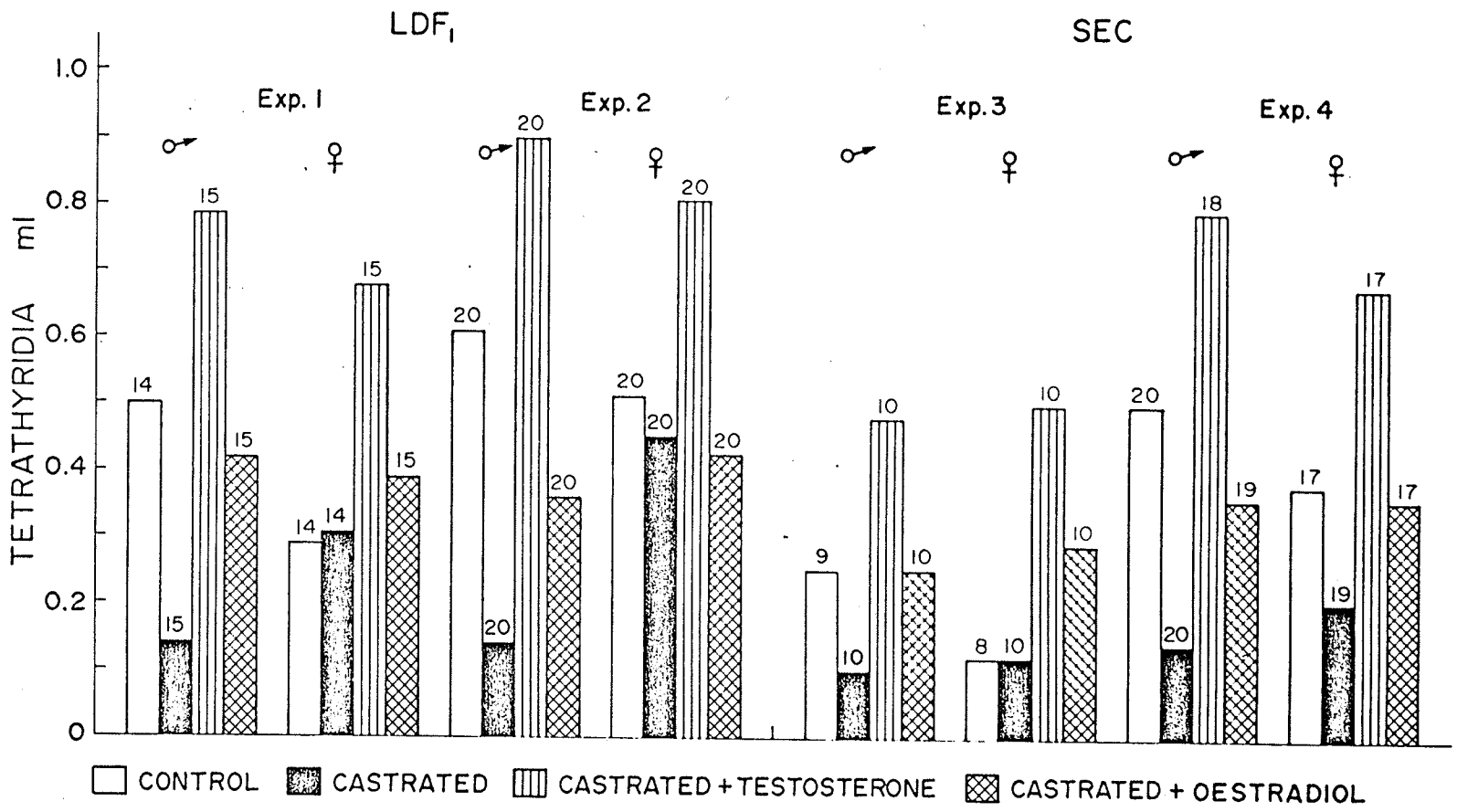

LDF1
SEC
Exp. 1
Exp. 2
Exp. 3
Exp. 4
TETRATHYRIDIA ml
1.0
0.8
0.6
0.4
0.2
0
14 15 15
14 14 15 15
20 20 20
20 20 20
9 10 10
8 10 10
20 20 18 19
17 19 17 17
CONTROL
CASTRATED
CASTRATED + TESTOSTERONE
CASTRATED + OESTRADIOL

Figure 2.

The size (as area) and the number os suckers of 300 tetrathyridia from populations of a control, a castrated, a castrated and testosterone treated, and a castrated and oestradiol treated LDF_1 male from experiment 2.

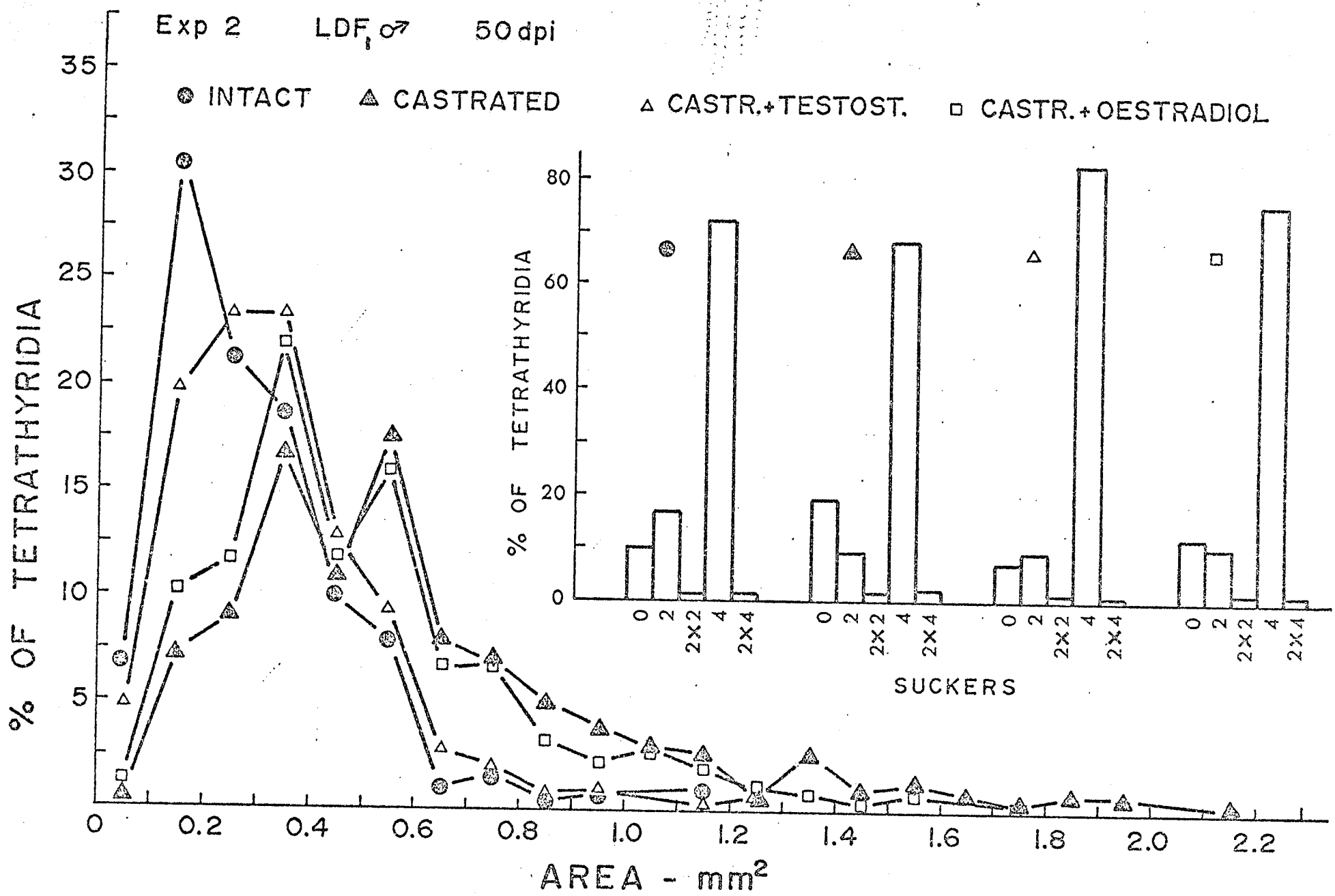

Exp 2 LDF1 ♂ 50 dpi
INTACT
CASTRATED
CASTR.+TESTOST.
CASTR.+OESTRADIOL
% OF TETRATHYRIDIA
AREA - mm²
% OF TETRATHYRIDIA
SUCKERS
0
2
2X2
4
2X4

TABLE 1.

Effect of gonadectomy and sex hormones upon liver weight and infection in LDF_1 and SEC mice, 50 days post infection.

Exp.	Sex	Age	No. of mice	Group	Liver weight, g (mean±S.E.)	Liver infection
1						
LDF_1	M	5	14	Control	3.73 ± 0.16	Moderate
			15	Castrated	2.72 ± 0.07	Very light
			15	Castrated+Testosterone	2.86 ± 0.07	Moderate-heavy
			15	Castrated+Oestradiol	4.39 ± 0.07	Heavy
	F	5	14	Control	3.03 ± 0.13	Light
			14	Castrated	3.25 ± 0.11	Light-moderate
			15	Castrated+Testosterone	2.09 ± 0.07	Moderate
			15	Castrated+Oestradiol	4.09 ± 0.14	Heavy
2						
LDF_1	M	8	20	Control	2.91 ± 0.07	Moderate
			20	Castrated	2.93 ± 0.03	Very light
			20	Castrated+Testosterone	2.95 ± 0.05	Moderate-heavy
			20	Castrated+Oestradiol	4.49 ± 0.07	Heavy
	F	8	20	Control	2.31 ± 0.06	Light
			20	Castrated	2.61 ± 0.08	Light-moderate
			20	Castrated+Testosterone	2.48 ± 0.06	Moderate
			20	Castrated+Oestradiol	4.36 ± 0.09	Heavy

3						
SEC	M	4	9	Control	2.75 ± 0.10	Moderate
			10	Castrated	2.57 ± 0.09	Very light
			10	Castrated+Testosterone	2.66 ± 0.11	Moderate-heavy
			10	Castrated+Oestradiol	3.13 ± 0.13	Heavy
	F	4	8	Control	2.46 ± 0.09	Light
			10	Castrated	2.75 ± 0.10	Light-moderate
			10	Castrated+Testosterone	2.68 ± 0.10	Moderate
			10	Castrated+Oestradiol	2.98 ± 0.12	Heavy
4						
SEC	M	3	20	Control	2.74 ± 0.07	Moderate
			20	Castrated	2.45 ± 0.08	Very light
			18	Castrated+Testosterone	2.95 ± 0.13	Moderate-heavy
			19	Castrated+Oestradiol	3.09 ± 0.08	Heavy
	F	3	17	Control	2.42 ± 0.08	Light
			19	Castrated	2.53 ± 0.07	Light-moderate
			17	Castrated+Testosterone	2.93 ± 0.11	Moderate
			17	Castrated+Oestradiol	3.09 ± 0.09	Heavy

APPENDIX

Figure 3.

Castration of male mice

A. Skin and musculature cut; testes brought through the opening;

B. testes removed and incision through musculature sutured;

C. skin wound closed with wound clip.

3A

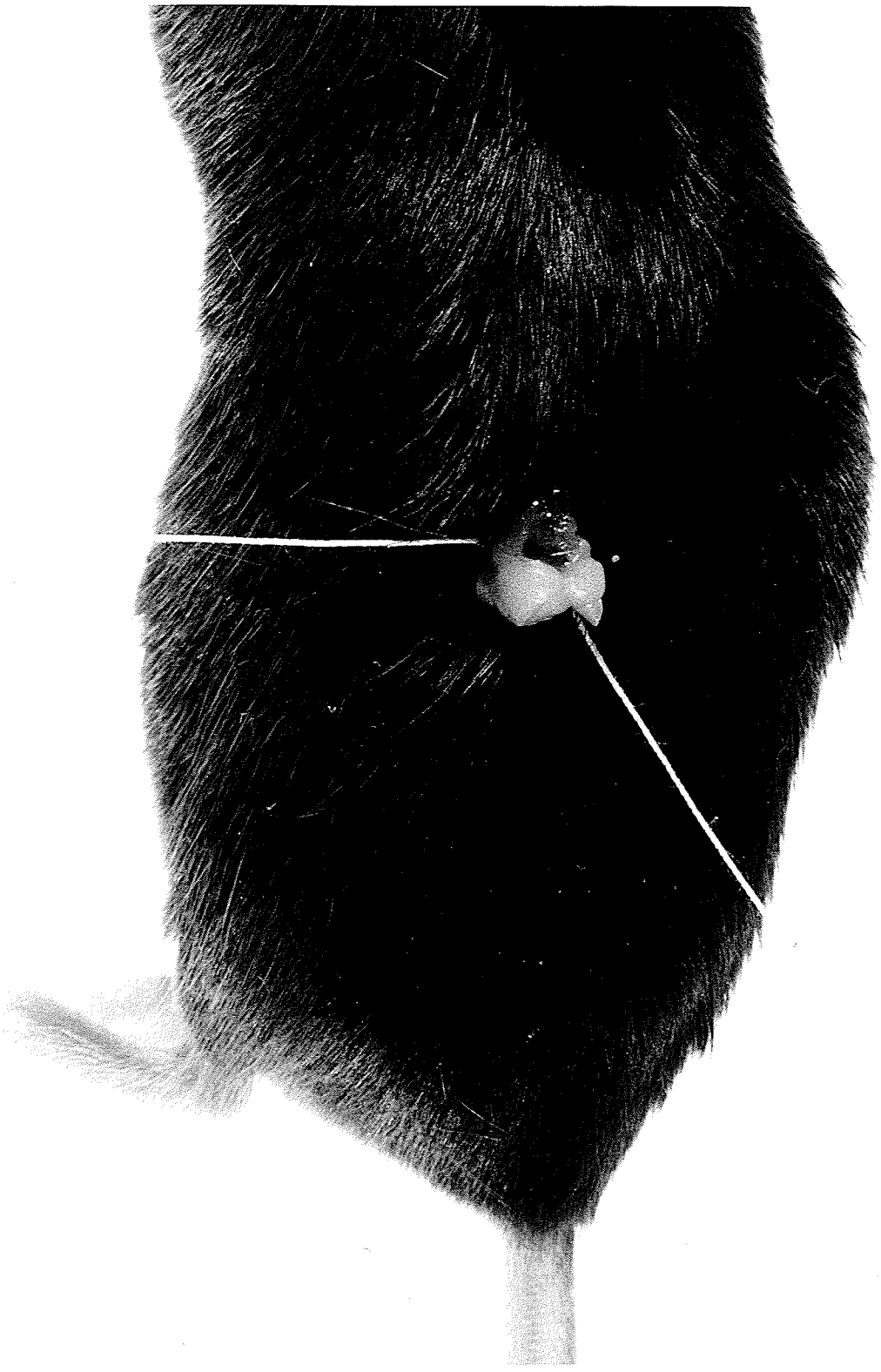
3B

Figure 4.

Castration of female mice

A. Incision through skin and musculature; ovary brought through the opening and tied off;

B. Ovary removed; incision through musculature sutured; skin wound closed with wound clip.



4A

4B

PART III.

ACCELERATION OF THE GROWTH OF TETRATHYRIDIAL POPULATIONS OF *MESOCESTOIDES CORTI* (CESTODA : CYCLOPHYLLIDEA) BY SPLENECTOMY.

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(1)

Mesocestoides tetrathyridia and splenectomy

Abstract

Experiments with LDF_1 hybrid mice showed that splenectomy 7 days prior to infection, increases the total biomass of tetrathyridial populations of *Mesocestoides corti* in hosts of both sexes.

This increase is accompanied by a decrease in the size of individuals, and by an increase in the percentage of two-suckered and acephalic forms. Splenectomy thus accelerates both the growth of the biomass of populations and the asexual multiplication of the tetrathyridia.

INDEX KEY WORDS: *Mesocestoides corti*, tetrathyridia, LDF_1 hybrid mice, splenectomy, biomass of larval population.

INTRODUCTION

THE EFFECT of splenectomy on the course of protozoan infections has been studied since the end of the last century, when Plimmer and Bradford (1899) published their paper on experimental trypanosomiasis. The role of spleen removal on the course of experimental malaria was studied by Gonder and Rodenwaldt (1910) and this direction of research developed by many recent authors. Primates, including man, are normally refractory to babesial infection; splenectomy renders them susceptible (Skrabalo and Deanović, 1957; Garnham and Bray, 1959).

Bittner (1913) showed that splenectomy has little effect on the course of experimental trichinellosis in rabbits. Negative results were also obtained by many other authors working with *Nippostrongylus brasiliensis* (Ogilvie and Jones, 1967), *Aspiculuris tetraptera* (Mathies, 1962), and other nematodes. However, splenectomy increases the numbers of *Loa loa* microfilariae in monkeys (Duke, 1960) and of those of *Litomosoides carinii* in cotton rats (Hawking, 1962).

Bruce *et al.* (1966) demonstrated that splenectomized *Rhesus* monkeys infected with *Schistosoma mansoni* pass more eggs than the controls and for a longer period of time.

Data on the role of splenectomy in infections with larval cestodes are scarce. Hoeppli in 1941 showed that splenectomy increased the number of developing strobilocerci of *Hydatigera taeniaeformis* in mice. Larsh (1944) pointed out that adult mice, splenectomized when young, were more susceptible to infections with *Hymenolepis nana* than control mice. On the other hand Lee (1970) experimenting with large numbers of LDF_1 and $B6D2F_1$ hybrid mice, found that splenectomy does not accelerate the growth of *Echinococcus multilocularis* cysts.

The present study investigates the influence of splenectomy on the growth of the total biomass of populations of *Mesocestoides corti* Hoeppli, 1925 tetrathyridia, and on the morphology of the individuals composing these populations.

MATERIALS AND METHODS

First generation LDF_1 hybrid mice (C57L females x DBA_1 males), 4 months old, of both sexes and of matched weights were used. They were kept, five per cage, at a temperature of 20°C and humidity of 50%, and fed Purina Lab chow *ad libitum*. Light cycle was 15 h simulated daylight and 9 h darkness.

Splenectomy was performed one week before infection. Each mouse was injected intraperitoneally with Avertin (Winthrop) 1% aqueous solution prewarmed to 40°C, 100 mg/kg body weight. Narcosis lasted for at least 30 minutes. The animal was then placed on its right side and the left was cleaned with 80% alcohol. A 0.5 cm long dorso-ventral incision through skin and musculature was made 0.5 cm posterior to last rib and the spleen extracted. The splenic ligament with its vessels was ligated and the spleen cut off. Incisions were sutured using circle needle No. 18 and surgical silk Ethicon, 000.

In sham-operated mice the operation was the same, but the splenic ligament was not ligated and the spleen not removed. There was no postoperative mortality.

As a source of tetrathyridia served a strain, received from Prof. M. Voge, University of California, Los Angeles, in August 1968, inoculated here into C57L female x DBA_1 male hybrid mice, and serially transferred every 3 months. Mice were infected intraperitoneally with 0.3 ml of sedimented tetrathyridia (200-300 individuals) of *M. corti*. All animals were autopsied 50 days post infection and the volumes of intraperitoneal larval populations

were determined by letting them sediment in a tuberculin syringe.

The areas of 200 larvae from control and splenectomized mice of both sexes were measured with a compensating planimeter, following the method of Voge and Coulombe (1966). Larvae were classified by the number of scolices (or their absence), and by the number of suckers per scolex as follows: acephalic fragments (designated as "0"), larvae with two suckers only (as "2"), larvae with two scolices, each with 2 suckers (as "2x2"), larvae with a single scolex with 4 suckers (as "4"), and larvae with two fully developed scolices (as "2x4").

Group means and their standard errors were calculated. Student's t-test was used for statistical analyses. Probability levels greater than 5 per cent were considered as statistically not significant.

RESULTS

Two experiments involving a total of 220 mice were made. In the first one 50 LDF_1 mice of each sex were divided into an experimental (splenectomized) and a control group of 25 animals each and infected simultaneously as described above. The total volumes of populations of tetrathyridia recovered at autopsy 50 days after infection are summarized in Table 1. In both males and females splenectomy increased the total volume of population by 9 per cent and 12 per cent respectively, the difference in both cases being statistically significant.

In the second experiment, involving 120 mice, 60 mice of each sex were divided into 3 equal groups of controls, sham-operated and splenectomized animals. All mice were infected simultaneously one week after the operation and dissected 50 days later.

There was practically no difference between the average volumes of populations from control and sham-operated animals (Table 1). But the total volumes of populations from splenectomized animals exceeded those from controls by about 40 per cent in males and 35 per cent in females, the differences being statistically significant.

In all mice tetrathyridia penetrated into the livers, but the intensity of invasion of this organ was not different in the variously treated groups of animals.

The morphological polymorphism of the larval populations was studied in the second experiment, in measuring surface areas of 200 tetrathyridia from each population and determining the mean and its standard error. The larvae from control males had a mean area of 0.18 ± 0.01 mm^2, and those from splenectomized males, 0.15 ± 0.01 mm^2. The corresponding figures for females were 0.20 ± 0.01 and 0.17 ± 0.01 mm^2. The smaller size of tetrathyridia from splenectomized animals was always correlated with an increase in the percentage of immature larvae (with two suckers), and often with an increase in the percentage of acephalic forms (Fig. 1).

Of particular significance may be the difference in numerical relation between larvae under 0.1 mm^2 in size, and those from 0.2 to 0.3 mm^2.
In male and female control mice the larger larvae were more common than the smaller, whereas in the splenectomized mice of both sexes this relation was reversed.

Populations from both male and female control mice contained a few large larvae with areas of 0.8 to 1.2 mm^2 and with many lateral stumps, remaining after the separation of daughter larvae (see Novak, 1972, Fig. 1). Such larvae were not found in populations from splenectomized hosts.

DISCUSSION

The removal of the spleen, an important source of antigen-reactive cells, accelerated the growth and multiplication of tetrathyridial populations. As result the average size of larvae decreased and the percentage of immature larvae and acephalic forms increased. Thus splenectomy increased the total biomass of larval populations and changed their composition in a way similar to that of the cytostatic agents cyclophosphamide and dactinomycin, and the parasiticides quinacrine and lucanthone (Novak and Lubinsky, 1973).

The role of spleen in immunological response to *Hymenolepis nana* infection was studied by Friedberg *et al.* (1967) by transplanting spleen cells from mice immunized by egg infection to irradiated recipients, which were later challenged with worm eggs. At autopsy the recipients had significantly lower numbers of cysticercoids than nonimmunized animals. The immunity was thus transferred by transplanted spleen cells.

That mice develop protective immunity against *Mesocestoides* larvae was recently reported by Kowalski and Thorson (1972a). These authors incubated tetrathyridia in sera of mice with tetrathyridial infections and observed the formation of a precipitate around the larvae.

In another experiment immune serum was injected intraperitoneally into infected mice. At autopsy the treated animals harbored less larvae than the controls. In another paper (1972b) these authors produced immunity in mice using secretions and excretions of tetrathyridia as antigens.

The present experiments showed that splenectomy accelerates both the growth and the multiplication of tetrathyridial populations: the larger populations from splenectomized mice consist of smaller individuals than those from the control mice.

ACKNOWLEDGMENTS

This research was supported by the National Research Council of Canada Grant A-1426 to Professor George Lubinsky.

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Figure 1.

The size (as area) and the polymorphism of tetrathyridia in larval populations from control and splenectomized LDF_1 mice of both sexes.

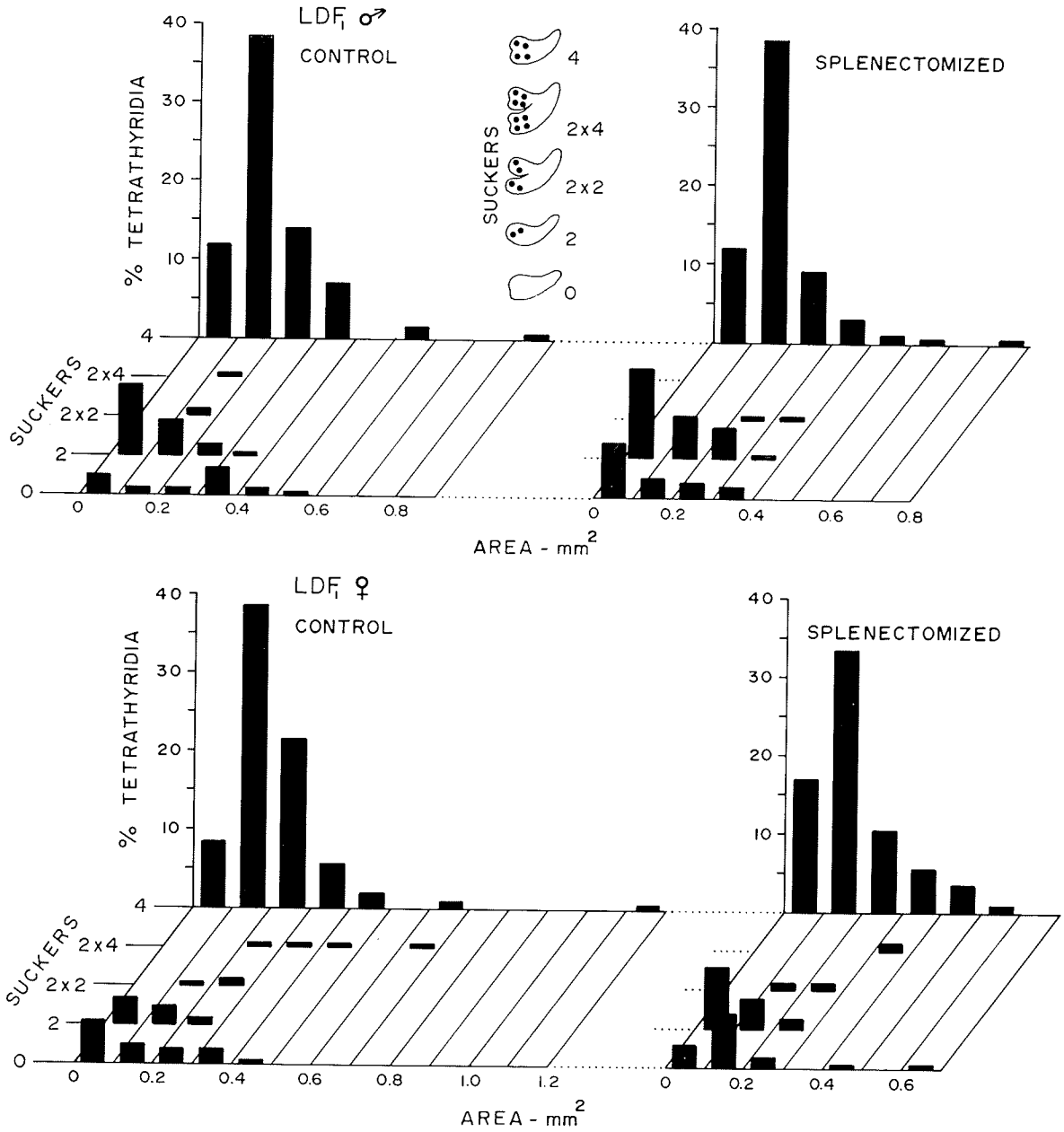
LDF1 ♂
CONTROL
SPLENECTOMIZED
% TETRATHYRIDIA
SUCKERS
4
2x4
2x2
2
0
AREA - mm2
LDF1 ♀
CONTROL
SPLENECTOMIZED
% TETRATHYRIDIA
SUCKERS
AREA - mm2

TABLE 1 - THE VOLUMES OF BIOMASS OF TETRATHYRIDIAL POPULATIONS OF *M. corti* IN CONTROL, SPLENECTOMIZED AND SHAM-OPERATED LDF_1 MICE, 50 DAYS POST INFECTION

Group	No. of Mice	Sex	Population volume, ml (mean + S.E.)	P (Probability levels)
Exp. 1				
Control	25	M	0.44 ± 0.01	-
Splenectomized	25	M	0.48 ± 0.02	< 0.05
Control	25	F	0.42 ± 0.01	-
Splenectomized	25	F	0.47 ± 0.01	< 0.01
Exp. 2				
Control	20	M	0.37 ± 0.01	-
Sham-operated	20	M	0.34 ± 0.01	-
Splenectomized	20	M	0.52 ± 0.01	< 0.001
Control	20	F	0.31 ± 0.01	-
Sham-operated	20	F	0.32 ± 0.01	-
Splenectomized	20	F	0.42 ± 0.01	< 0.001

APPENDIX

Figure 2.

Splenectomy

A. Incision of the left side of the abdominal wall; ligation of splenic ligament and removal of spleen.

B. Suturing of musculature is followed by closing the skin incision with wound clip.

2A

2B

PART IV.

CORTISONE AND THE GROWTH OF POPULATIONS OF *MESOCESTOIDES* TETRATHYRIDIA IN MICE

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ABSTRACT

Volumes and weights of populations of Mesocestoides tetrathyridia increased faster in SEC mice treated with cortisone, than in the controls. Cortisone did not alter the sex resistance of the host and the populations always grew faster in male than in female hosts. Larvae of the populations from cortisone treated mice were smaller than those from control mice, and those from males smaller than those from females. The larger the populations, the smaller was the average size of the larvae. Fast growing populations contained a higher percentage of larvae with 2 suckers. These forms can probably serve as indicators of accelerated splitting of the larvae.

INDEX KEY WORDS: Mesocestoides corti, tetrathyridial populations, cortisone.

INTRODUCTION

IN a previous paper Novak (1974a) showed that splenectomy, 7 days prior to infection, accelerated both the growth of larval populations and the asexual multiplication of tetrathyridia of Mesocestoides corti in mice. In the present study cortisone was used to suppress the resistance of mice to the growth of tetrathyridial populations.

MATERIALS AND METHODS

Forty SEC mice, 4 months old, of each sex and of matched weights were used in each experiment. They were infected with tetrathyridia and divided into control and cortisone treated groups. Treatment with cortisone acetate (BDH Biochemicals Ltd., Poole, England) was started one day after infection. Cortisone, 10 µg/g, was injected subcutaneously twice a week, in alternating 3 and 4 days intervals, and a total 10 injections given. Mice were autopsied 50 days post infection. The second experiment was an exact replication of the first one.

The techniques of infecting mice, of measuring the volumes of sedimented larval populations, of evaluation of liver infections, and of classification of larvae were described earlier (Novak, 1972, 1974a & 1974b).

The wet and the dry weights of populations from each mouse were recorded. Larvae were blotted with filter paper, weighed, dryed in an oven at 60°C for 12 h, and weighed again.

Two hundred tetrathyridia were taken at random from populations of one control and of one cortisone treated mouse of each sex and the body areas of the larvae measured. The mean area of larvae of each population and the corresponding standard error were determined.

Student t-test was used for analysis of data. Probability levels greater than 5% were not considered statistically significant.

RESULTS

Fig. 1 shows the volumes of tetrathyridial populations from control and cortisone treated SEC mice. In both experiments control males had significantly larger populations than control females, with $P < 0.01$ and $P < 0.001$ in experiment 1 and 2, respectively. The volumes of intraperitoneal populations in male mice treated with cortisone increased in experiment 1 by 171% and in experiment 2 by 120%, and in females by 154 and 71%, respectively. All differences were significant ($P < 0.001$).

To estimate the biomass of the larval populations their wet and dry weights for each mouse were recorded (Fig. 2). The difference in wet weight between populations from control males and cortisone treated males was 215% in experiment 1, and 170% in experiment 2.

The corresponding figures for the females were 239 and 92% respectively. The difference in dry weight between populations from control and cortisone treated mice was 205 and 286% in males and 156 and 141% in females respectively. All differences were statistically significant ($P < 0.001$). The mean difference between wet and dry weight of all populations was 74 ± 4 %.

The morphological polymorphism of tetrathyridia was studied in larval populations of a single mouse from each group of experiment 2. Larvae from a control male had a mean area of 0.23 ± 0.01 mm^2, those from a cortisone treated male, 0.15 ± 0.01 mm^2. Larvae from a control female had a mean area of 0.43 ± 0.02 mm^2, those from a cortisone treated female, 0.30 ± 0.02 mm^2. Thus larvae from treated mice were considerably smaller than those from controls. The size of larvae in different groups decreased in the following order: control females > control males > cortisone treated females > cortisone treated males.

The majority of larvae from the population of the cortisone treated male had areas from 0.1 to 0.3 mm^2; the largest larva had an area of 0.6 mm^2. Larvae from the population of the control female were more evenly distributed among size groups, and the largest one had an area of 1.7 mm^2. Larvae from the control male and the cortisone treated female were intermediate in size (Fig. 3). There was a higher percentage of acephalic forms ("0") and of larvae with split scolices and 4 suckers each ("2x4") in control mice than in the cortisone treated mice, especially in the female. In the large populations from the cortisone treated mice, the

relations were reversed, and there was a considerable increase in percentage of larvae with 2 suckers.

The intensity of invasion of livers was higher in cortisone treated mice than in the controls. Whereas the livers of control males were "moderately" infected, and those of control females "lightly", in treated males the infection was "moderate-heavy", and in females "light-moderate".

DISCUSSION

Research on the effects of cortisone on adult cestode infections has centred on Hymenolepis. As a rule, cortisone lowered the resistance of the host and permitted better growth and establishment of worms in the host's intestine (Weinman & Rothman, 1961; Moss, 1972; Hopkins et al. 1972).

Olivier (1962), Esch (1964 & 1967), and Lee (1970) studied the effect of cortisone on infections with larval cestodes. Olivier used cortisone to break down the natural resistance of mice to Taenia taeniaeformis . Esch showed that cortisone increased the number of Multiceps serialis larvae in subcutaneous tissues of mice, but not in the central nervous system. Lee, working with LDF_1 hybrid mice, found that cortisone considerably accelerated the growth of cysts of Echinococcus multilocularis.

The present study shows that cortisone has a similar effect on larval populations of M. corti. The volumes of these populations were larger in cortisone treated mice than in controls. Cortisone did not alter the sex resistance of the host: populations were always larger in males, than in females, whether cortisone treated or controls.

Larvae from populations of cortisone treated mice were smaller than those from control mice, larvae from males smaller than those from females. Acceleration of the splitting process in cortisone treated mice was correlated with a higher percentage of prematurely separeated larvae, those with only 2 suckers.

Both the mean wet and dry weight of one ml of sedimented larval populations were higher in cortisone treated mice than in controls (Table 1). This was observed in both experiments and in respect of hosts of both sexes. This is probably due to the smaller average size of individuals in the larger populations from cortisone treated hosts, though other factors such as differences in the specific gravity of larvae may also play a role.

Cortisone, unlike oestradiol (Novak, 1973), increased only slightly the infection of liver, the most heavily infected organ of the host.

The increase of the volumes and of the weight of Mesocestoides populations in cortisone treated mice may be due to (1) a suppression of immunity by this hormone, or (2) a direct effect of the hormone on the larvae, or (3) a combination of both these factors. Though the effect of the splenectomy on the tetrathyridial populations

(Novak, 1974a) supports the first hypothesis, a study of the effects of cortisone and of other steroid hormones on larval populations *in vitro* may contribute to the explanation of the phenomena observed in this work.

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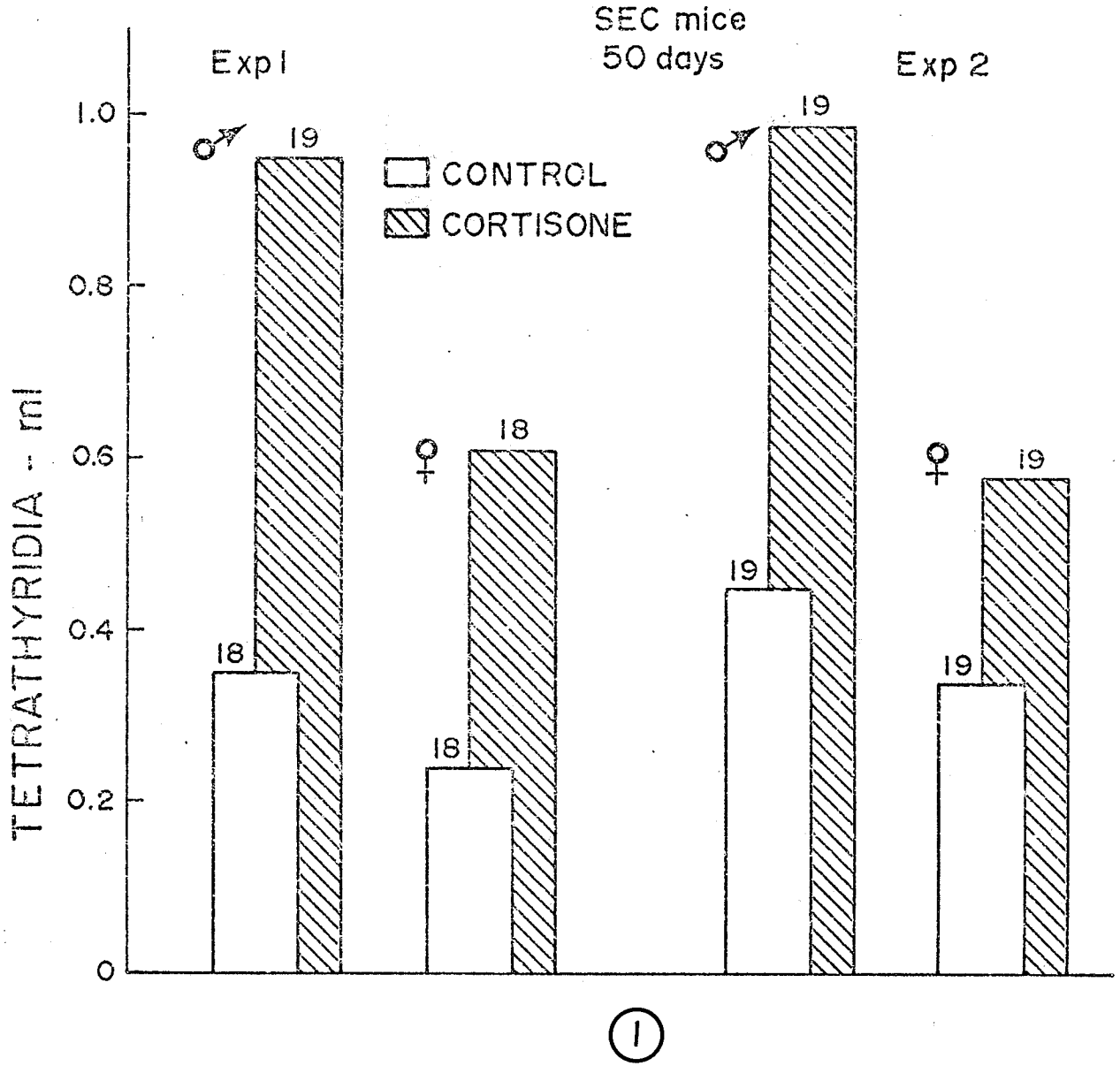
Figure 1.

Mean volumes of tetrathyridial populations in control and cortisone treated SEC mice from experiments 1 and 2. The numbers above the bars represent the number of animals used.


SEC mice
50 days
Exp 1
Exp 2
CONTROL
CORTISONE
TETRATHYRIDIA - ml
1.0
0.8
0.6
0.4
0.2
0
♂
♀
18
19

1

Figure 2.

Mean wet and dry weight of tetrathyridial populations in control and cortisone treated SEC mice from experiment 1 and 2. The numbers above the bars represent the number of animals used.

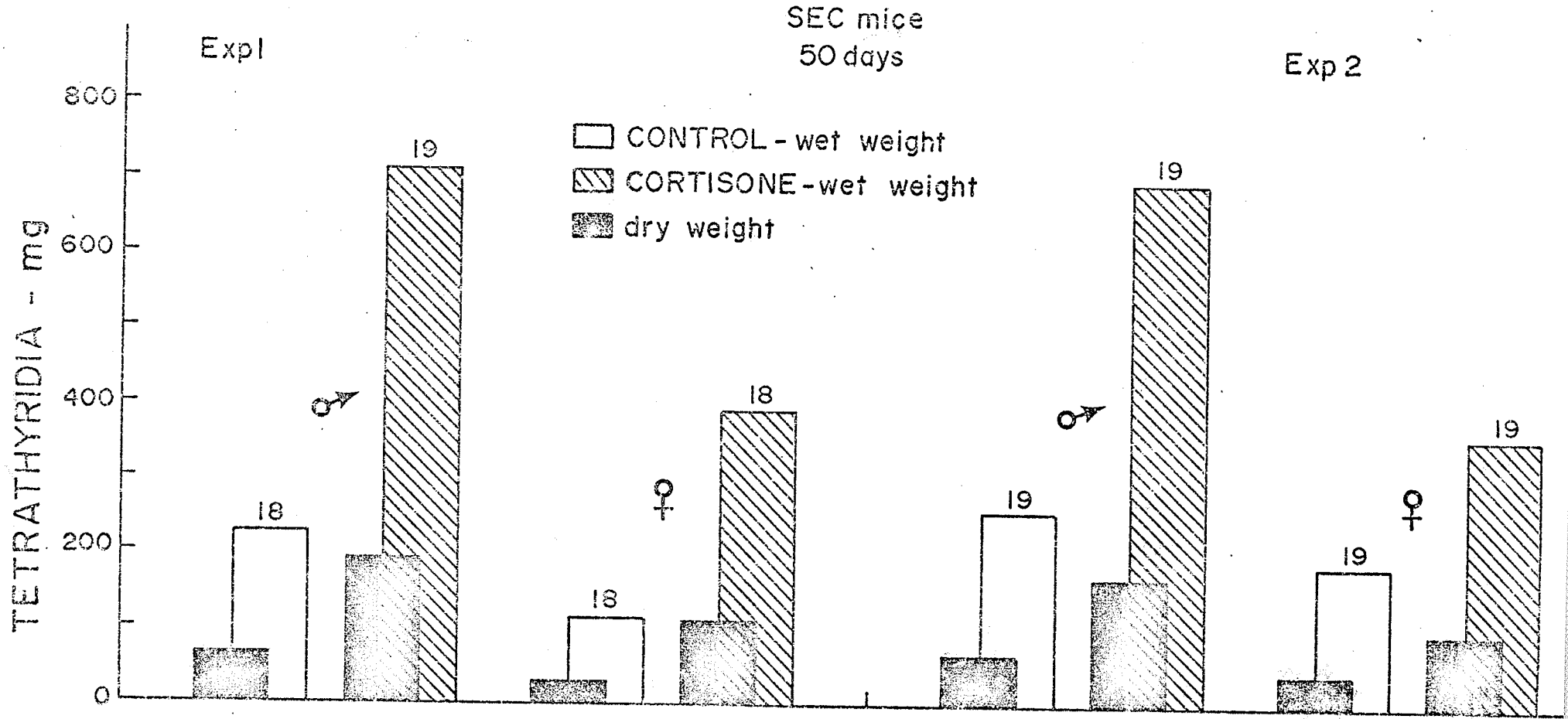

SEC mice
50 days
Exp1
Exp 2
CONTROL - wet weight
CORTISONE - wet weight
dry weight
TETRATHYRIDIA - mg
800
600
400
200
0
♂
♀
♂
♀
18
19
18
18
19
19
19
19

Figure 3.

The size (as area) and the number of suckers of 200 tetrathyridia in populations from control and cortisone treated SEC mice of both sexes from experiment 2.

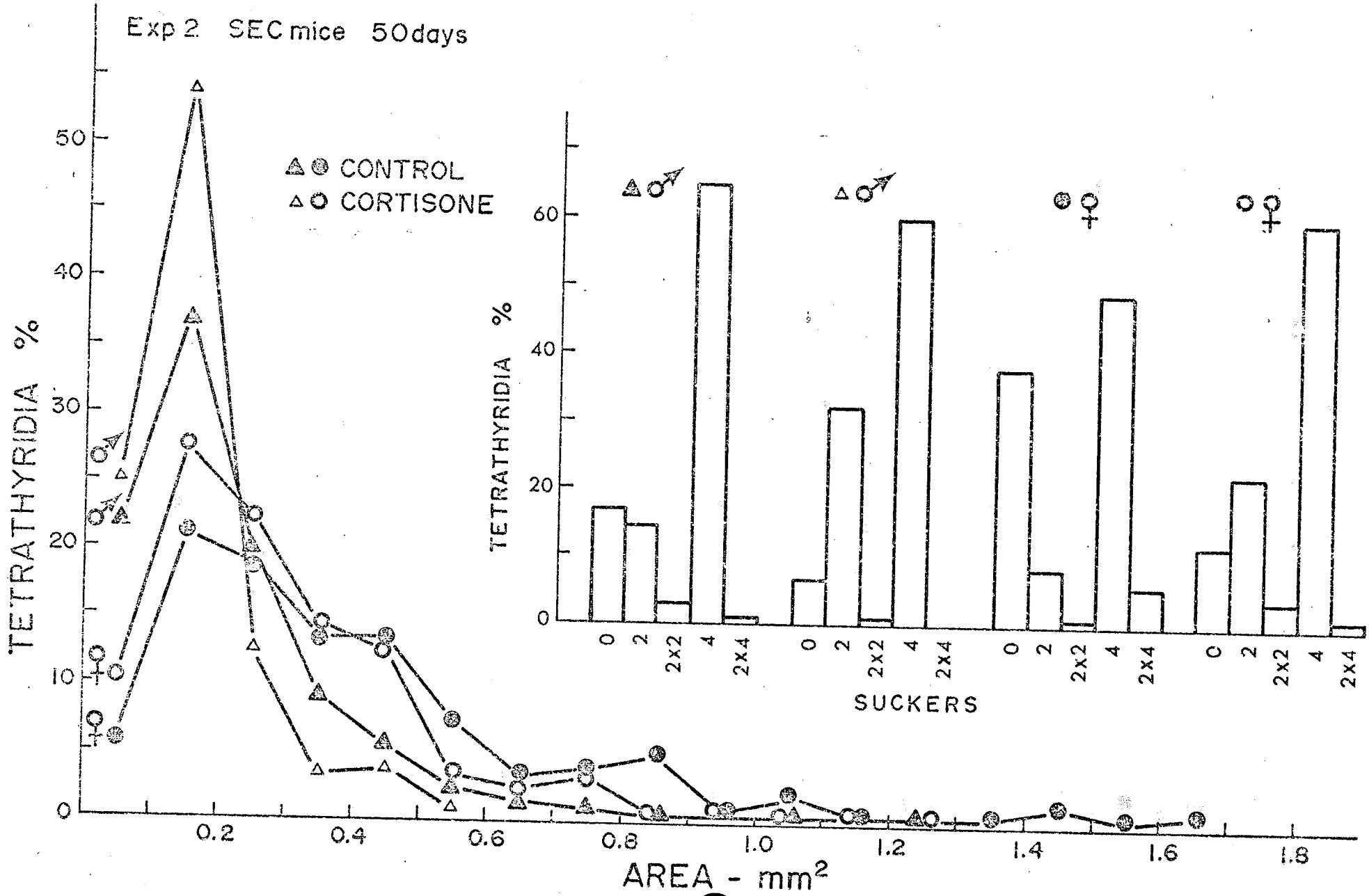

Exp 2 SEC mice 50days
CONTROL
CORTISONE
TETRATHYRIDIA %
AREA - mm²
0
10
20
30
40
50
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
TETRATHYRIDIA %
0
20
40
60
0
2
2x2
4
2x4
SUCKERS

TABLE 1

Mean wet and dry weight of larval populations per ml of sedimented population from control and cortisone treated SEC mice.

Group	Sex	No. of mice	Wet weight g/ml	Dry weight g/ml
Exp. 1.				
Control	M	18	0.645	0.180
Cortisone	M	19	0.748	0.202
Control	F	18	0.479	0.121
Cortisone	F	18	0.640	0.183
Exp. 2.				
Control	M	19	0.571	0.147
Cortisone	M	19	0.701	0.171
Control	F	19	0.550	0.121
Cortisone	F	19	0.619	0.171

CONCLUSIONS

SUMMARY

1. The volumes of tetrathyridial populations of Mesocestoides corti in the peritoneal cavities of mice increase exponentially with time. Thirty days post infection more larvae are in these cavities than in the livers. After that the increase in the volumes of intraperitoneal populations continues, at least up to 60 days post infection, yet the liver infection remains constant.
2. Testosterone propionate, 10 µg/g x 10, increases significantly the volumes of intraperitoneal populations in intact mice of both sexes.
3. Oestradiol benzoate, 5 or 10 µg/g x 10, increases the volumes of intraperitoneal populations in mice of both sexes much less than testosterone. A larger dose of oestradiol, 10 µg/g x10, does not increase significantly the volumes of tetrathyridial populations above those in mice receiving the lower dose.
4. Oestradiol, 5 or 10 µg/g x 10, increases singificantly the invasion of livers with tetrathyridia.
5. Orchiectomy decreases the total volumes of intraperitoneal populations and increases the average size of tetrathyridia.
6. Testosterone, 10 µg/g x 10, increases the volumes of intraperitoneal populations in castrated males above that of intact males, and decreases the average size of tetrathyridia.
7. Oestradiol , 5 µg/g x 10, also increases the volumes of intra-

peritoneal populations in castrated males, but only to the level of those from intact males, and decreases slightly the average size of larvae.

8. Ovariectomy decreases the volumes of populations but slightly, and sometimes does not affect it at all.
9. Testosterone, 10 µg/g x 10, considerably increases the volumes of intraperitoneal populations in ovariectomized mice, often doubling it.
10. Oestradiol, 5 µg/g x 10, given to ovariectomized mice mostly does not affect the volumes of populations but sometimes slightly increases it.
11. Oestradiol, 5 µg/g x 10, increases significantly the liver invasion in castrated mice of both sexes.
12. Splenectomy 7 days prior to infection increases the total volumes of intraperitoneal populations in LDF_1 mice of both sexes.
13. This increase is accompanied by a decrease in the size of tetrathyridia and an increase in the percentage of larvae with two suckers and of acephalic forms.
14. Cortisone, 10 µg/g x 10, increases significantly the volumes of tetrathyridial populations in SEC mice of both sexes.
15. Cortisone increases significantly both the wet and dry weight of tetrathyridial populations in SEC mice of both sexes, and more so in male than in female hosts.
16. Larvae from the populations of cortisone treated mice are smaller than those from control mice, those from males smaller than those from females.

GENERAL CONCLUSIONS

1. All three hormones studied in the present paper, namely oestradiol, testosterone, and cortisone, increase the volumes of intraperitoneal populations of Mesocestoides tetrathyridia, their efficiency increasing in the above order.
2. Splenectomy also increases the volumes of tetrathyridial populations and is approximately as efficient as oestradiol.
3. Oestradiol increases considerably the invasion of liver by tetrathyridia.
4. Gonadectomy is the sole known factor which decreases the volumes of intraperitoneal populations. Orchiectomy is more efficient than ovariectomy in this respect.

PLANS FOR FURTHER RESEARCH

Further research on populations of Mesocestoides tetrathyridia may be developed along the following lines:

1. In vitro studies may answer the question wheather the effect of steroid hormones or of chemotherapeutic agents on the growth of tetrathyridial populations is direct or host-mediated. (Kowalski and Thorson (1972) showed that cholesterol and some other lipids stimulate the growth and asexual reproduction of tetrathyridia in vitro).
2. Why larvae invade heavily the livers of mice treated with oestradiol? Is this caused by this hormone as such or by its derivatives (e.g. oestron or oestriol) to which it is converted in the liver?
3. Slower growth of tetrathyridial populations in castrated hosts and the relative frequency of occurence of polycephalic forms in slow growing populations suggests a study of the development of these forms in experiments of long duration with castrated animals (Fig. 1, 2).
4. The effects of splenectomy and cortisone on the tetrathyridial populations suggest that both the cellular and humoral immune responses of the host control the parasite load. Immunological studies are necessary to understand the host-parasite relationship in infections with tetrathyridia.
5. It may be interesting to study in vivo the interrelations between

the tetrathyridial populations and those of other larval cestodes, especially of larvae multiplying asexually. Such studies may reveal theoretically and practically important immunological interrelations between these parasites and the host.

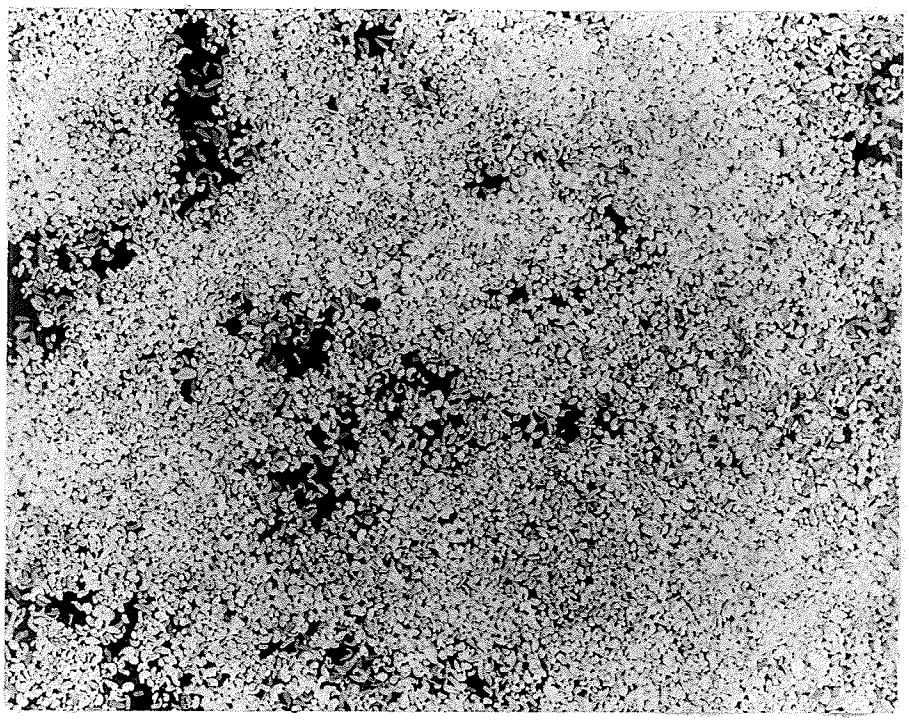
These are only a few of the many lines of research which can be based on the finding reported in this thesis.

Figure 1.

Populations of tetrathyridia after 50 days of infection. No polycephalic forms are present, x 2.

Figure 2.

Polycephalic larvae selected from populations after 150 days, x 2.



1

2

APPENDIX

TAXONOMY

(after Wardle and McLeod, 1952)

Phylum	Platyhelminthes	Carus, 1863
Class	Cestoda	Monticelli, 1892
Subclass	Eucestoda	Southwell, 1930
Order	Cyclophyllidea	Braun, 1900
Family	Mesocestoididae	Perrier, 1897
Genus	*Mesocestoides*	Vaillant, 1863
Species	*corti*	Hoeppli, 1925

HISTORY OF RESEARCH ON *MESOCESTOIDES* TETRATHYRIDIA

The adult cestode was described by Hoeppli (1925) from *Mus musculus* collected by W.W. Cort in 1909 at Colorado Springs. This material consisted of about a hundred adult specimens from the intestine of this host.

The development and morphology of the oncosphere of *M. corti* was studied by Ogren (1955). The structure of developmental stages from hatched oncosphere to first larval stage - procercoid-like, is known only from *in vitro* studies (Voge, 1967; Voge and Seidel, 1968) and remains to be found in nature.

The second larval stage - tetrathyridium - is found in lizards and rodents. The name "Tetrathyridium" was proposed as a generic name by Rudolphi in 1819. Later Leuckart (1874) and Moniez (1880) showed that these organisms are developmental stages of *Mesocestoides*. The body of this solid plerocercoid is filled with parenchyma containing numerous calcareous corpuscles. The crystalization patterns and variations in size and shape of these corpuscles in adult and larval forms were studied by von Brand *et al.*(1969). The irregularly shaped corpuscles of the larval and the adult *M. corti* appear to be identical.

The nerve, muscular and excretory systems of tetrathyridia were described by Hart (1968a) Histologically these larvae are very similar to those of *Taenia* and *Multiceps* (Voge and Berntzen, 1963).

Peculiar to the tetrathyridium of M. corti is its capability to multiply in laboratory rodents by longitudinal fission (Specht and Voge, 1965). Details of this process were described by Voge (1969), Hart (1967), Hess (1972), and Novak (1972). The anterior halves of transected larvae have the ability to regenerate or even strobilate, but posterior ends survive only for some time and then degenerate (Hart, 1968). Eckert, von Brandt and Voge (1969) reported asexual multiplication of larvae of M. corti in the intestine of the definitive hosts, dogs and skunks.

Tetrathyridia are highly pathogenic to their mammalian hosts. In dogs fatal parasitic ascites and chronic peritonitis were reported (Voge and Berntzen, 1963). In rodents, liver is always the most infected organ. This predilection may depend on the necessity of blood as a source of nutrition. Voge and Coulombe (1966), and Voge and Seidel (1968) found that tetrathyridia do not multiply in media without whole blood.

The pathology of mouse liver infections with tetrathyridia of M. corti was studied by Specht and Widmer (1972). Acute inflammation and extensive anoxic hepatocyte necrosis occured, followed by massive parenchymal regeneration. Subsequent encapsulation of tetrathyridia was observed with infiltration of eosinophils and plasma cells. In old infections portal lymphatic obstruction and ascites were often present. Lungs, muscles and sex organs also become invaded in older infections (Novak, 1972 a)

Kowalski and Thorson (1972a,b) demonstrated the existence of passive immunity against Mesocestoides tetrathyridia both in vivo

and *in vitro*. Injections of the immune serum into mice lowered their parasite load. Larvae incubated with immune serum were surrounded by a precipitate formed by the reactions between their excretions and/or secretions and the antibodies.

Whereas passive immunity can to some extent protect the host against tetrathyridia, the cytostatic agents cyclophosphamide and dactinomycin, and the parasiticides lucanthone and quinacrine accelerate the asexual multiplication of larvae and increase the volumes of intraperitoneal populations in mice (Novak and Lubinsky, 1973).

Respiration of the larvae of *M. corti* was studied by Weinbach and Eckert (1969). Tetrathyridia were insensitive to changes in the oxygen tension from 100 to 5 vol. %, but the respiration was significantly diminished at a tension of 2 %. Thus they have basically an aerobic metabolism. The work of Voge and Coulombe (1966) demonstrated that tetrathyridia of *M. corti* multiplied better in cultures containing air than in cultures without it. Another indication that aerobic metabolism may be more important to these larvae than hitherto thought is the presence of typical mitochondria with double membranes and cristae.

Tetrathyridia, maintained *in vitro* in a synthetic medium containing preformed purines and pyrimidines, fail to synthetize these compounds *de novo* (Heath and Hart, 1970). However, if larvae are placed in a medium lacking these compounds, the incorporation of labeled orotic acid into nucleic acid pyrimidines is observed and so is the synthesis of purines.

It seems thus that the fast multiplying tetrathyridia of *M. corti* can serve as an excellent tool for biomedical research in various fields including pathology, physiology, immunology, biochemistry and chemotherapy.

REVIEW OF LITERATURE

SEX HORMONES AND HELMINTHIC INFECTIONS

Trematode infections

Lees and Bass (1960) reported the depression in the number of trematodes, "Polystoma integerrimum" (Polystomum integerrimum) and Gorgoderina vitelliloba in naturally infected Rana temporaria, by oestradiol benzoate.

Acanthocephalan infections

Oestradiol benzoate decreased the number of Acanthocephalus ranae in naturally infected Rana temporaria (Lees and Bass, 1960).

Cestode infections

Addis (1946) found that injections of theelin (an oestrogen) into female rats on vitamin deficient diet, produced a significant increase in the growth of adult Hymenolepis diminuta, though it failed to bring it to the level of that in rats on complete diets. Testosterone failed to produce normal growth of the worms in female rats on vitamin deficient diet. Bailenger et al. (1964) found, that the number of H. nana increased both in male and female mice treated with male sex hormone, but decreased in mice treated with oestrogen. Meyer and Valleau (1967) showed that testosterone given to male hamsters infected with Diphyllobothrium sebago increased the egg output of these worms.

Several reports on the influence of sex hormones on the cestode

larvae were published. Campbell (1939) showed, that the number of Taenia taeniaeformis cysticerci in theelin injected female albino rats did not differ from those of controls. The male rats which received theelin had less cysticerci than controls. Females receiving testosterone propionate had more cysts than the controls. Males injected with testosterone showed no change in this number. Both testosterone and oestradiol increased the growth rate of cysts of Echinococcus multilocularis in male and female mice (Baron, 1971). In the case of E. granulosus (Frayha et al.1971), testosterone increased the growth of the cysts in albino mice of both sexes, while oestradiol tended to decrease it.

Nematode infections

Ackert and Dewhirst (1950) showed that diethyl-stilbestrol administered to young chickens infected with Ascaridia galli lowered the worm counts. Methyl testosterone had no effect on worm counts, but it increased significantly the size of worms (Todd and Crowdus, 1951). According to Sadun (1951), male chickens which received testosterone, and females receiving oestradiol harboured smaller numbers of worms. The worms from treated males were larger, but those from treated females smaller than worms from the controls. Mathies (1959) found that testosterone had no effect on burdens of Aspicularis tetraptera in either male or female mice, but that β- oestradiol significantly lowered worm burdens in males.

Rohrbacher (1958 and 1960) found that stilbestrol pellets implanted into young rabbits had no effect on Trichostrongylus axei or T. colubriformis infections. The numbers of Rhabdias bufonis in naturally infected Rana temporaria can be decreased by oestradiol benzoate (Lees and Bass, 1960). Both female and male hamsters injected testosterone propionate had higher numbers of Nippostrongylus brasiliensis at necropsy than controls. But oestradiol benzoate had no effect (Solomon, 1966). Mankau and Hamilton (1972) showed the effect of sex hormones on the infection of hooded rats with Trichinella spiralis. Stilbestrol administered to male rats caused a decrease in the numbers of T. spiralis, but testosterone injected to females incerased it significantly.

Summary

Oestrogens depress the numbers of some trematodes, acanthocephalans, and of Rhabdias bufonis in frogs, and those of Hymenolepis nana and Aspiculuris tetraptera in mice. Oestrogens also depress the numbers of Trichinella spiralis in experimentally infected rats.

Oestrogens tend to decrease the number of strobilocerci of Taenia taeniaeformis in rats. Both oestrogens and testosterone accelerate the growth of cysts of Echinococcus multilocularis in mice. But the growth of E. granulosus cysts is inhibited by oestradiol and accelerated by testosterone.

Testosterone increases the egg output by Diphyllobothrium sebago in hamsters, and increases the numbers of Nippostrongylus brasiliensis in experimentally infected hamsters (higher percentage of larvae reach maturity).

CASTRATION OF THE HOST AND HELMINTHIC INFECTIONS

Trematode infections

Berg (1953) showed that castrated male albino mice harbored smaller numbers of male schistosomes than the controls.

Cestode imfections

Addis (1946) observed stunting of Hymenolepis diminuta caused by castration of male rats. The egg production by these worms was lower in gonadectomized and thyroidectomized hamsters than in intact hosts (Landt 1954, 1955). Landt and Goodchild (1962) found heavier adult H. diminuta in thyroidectomized and thyroidectomized+castrated rats of both sexes, than in controls. Beck (1952) observed depressed egg production by this tapeworm in orchiectomized rats. Aldrich et al.(1954) reported reduced activity of transaminase systems in adult H. diminuta from castrated male rats. Castration of the rat host caused a decline in the rate of glycogen synthesis by H. diminuta from both glucose and pyruvate (Daugherty, 1956).

Numbers of Cysticercus crassicollis increased in ovariectomized mice, but decreased in orchiectomized animals (Campbell and Melcher, 1940). Baron (1971) found that cysts of Echinococcus multilocularis grow slower in castrated mice of both sexes than in controls.

Nematode infections

Dobson (1961) found 5 days post infection more adult Nematospiroides dubius in castrated rats of both sexes than in controls, but 10 days later only the castrated males harbored more worms than the controls. Normally the worms were longer in females. In castrated females the worms were unchanged, but they were longer in castrated males than in controls (Dobson, 1966 b).

In laboratory mice infected with the third stage larvae of Amplicaecum robertsi the males have heavier infections than females and the worms are shorter in females. Castration abolished these differences (Dobson, 1966 a).

The mouse pinworm Aspicularis tetraptera infections were twice as heavy in females as in males. In castrated males the mean number of worms decreased by about 40%, whereas in castrated females it increased by about 20% (Mathies, 1954).

Solomon (1966) found that orchiectomized (but not the ovariectomized) hamsters harbored significantly lesser numbers of adult Nippostrongylus brasiliensis than the controls. Solomon and Haley (1968) stated that lower numbers of N. brasiliensis were found in orchiectomized rats and hamsters than in controls.

Dunsmore and Dudzinski (1968) found that female rabbits castrated during the breeding season had singificantly lower burdens of Trichostrongylus retortaeformis and Graphidium strigosum than the normal females. These loads were similar to those of the normal males. Salpingectomized females harbored fewer worms than normal females but more than the ovariectomized females and the

normal males.

Experiments with Strongyloides ratti showed no differences between worm burdens of gonadectomized and those sham-operated male and female rats (Katz, 1961).

Orchiectomy in jirds significantly reduced the numbers of both adult Brugia pahangi and of thier microfilariae. Ovariectomy had no effect (Wesley and Ash, 1972).

Summary

The review of literature shows that gonadectomy can influence the growth and reproduction of parasites, and the ability of the host to control both the parasites and their effects.

Castration of males depresses the number of male schistosomes in mice. Castration of male rats causes stunting of Hymenolepis diminuta and a depression of egg production by this cestode. Castration of male rats depresses the numbers of developing strobilocerci, whereas ovariectomy enhances it. Cysts of Echinococcus multilocularis grow slower in gonadectomized mice of both sexes.

In experimental imfections both with Amplicaecum robertsi and Nematospiroides dubius castrated males had more adult nematodes than the controls. Yet in Aspicularis tetraptera infections the mean numbers of worms decreased in castrated males, but increased in castrated females. Orchiectomized hamsters and rats had lesser numbers of Nippostrongylus braziliensis than the controls. Orchiectomy in jirds reduces the numbers of adult Brugia pahangi and of its

microfilariae.

In general castration of males tends to reduce the worm burdens of hosts, though there are some exceptions to this trend.

CASTRATION OF THE HOST, HORMONE TREATMENT AND HELMINTHIC INFECTIONS

Trematode infections

Berg (1957) showed that testosterone decreased the number of female Schistosoma mansoni in orchiectomized mice. On the other hand Robinson (1959) could not find any difference in survival rates of either sex of schistosomes between castrated mice treated with testosterone and the control animals.

Cestode infections

Adult cestodes

Addis (1946) found that orchiectomized rats given testosterone or methyl testosterone harbored normal Hymenolepis diminuta in contrast to castrated controls which had stunted worms. Theelin did not abolish this stunting. Neither testosterone nor theelin offset the effects of castration in the females. The drop in egg output of a singly-established H. diminuta caused by castration of the rat was brought back to normal by testosterone. Meyer and Valleau (1967) showed that testosterone given to castrated male hamsters infected with Diphyllobothrium sebago increases the egg output of these cestodes.

Larval cestodes

Testosterone increased the numbers of larval Taenia taeniaeformis in ovariectomized rats, but oestradiol decreased the numbers of larvae in orchiectomized rats (Campbell and Melcher, 1940). Both testosterone

and oestradiol increased the growth rate of Echinococcus multilocularis cysts in castrated mice of both sexes (Baron, 1971).

Nematode infections

Dobson (1961) found that testosterone did not decrease the number of Nematospiroides dubius in castrated male rats. Oestradiol increased the worm burdens of castrated females as compared to those of normal females. Later Dobson (1966 b) found that in 10 days old infections with this nematode in orchiectomized rats testosterone increased both the numbers of larvae penetrating into the gut wall and of those surviving to the adult stage. Oestradiol decreased the numbers of larvae which penetrated the intestinal wall in ovariectomized rats, and increased the resistance of castrated males. Dobson (1966 b) also found that testosterone had no effect on Amplicaecum robertsi infections in castrated mice of both sexes.

Katz (1963, 1967) showed that there was no difference in the total number of Strongyloides ratti in castrated rats of both sexes of either testosterone or oestrogen treated groups.

Testosterone increased the number of Nippostrongylus brasiliensis in castrated hamsters, and was more active in females than in males (Solomon, 1966).

Castrated male mice injected with stilbestrol (an oestrogen) harbored less Trichinella spiralis than normal males. Ovariectomized mice injected with testosterone harbored far more worms than normal females (Mankau and Hamilton, 1971).

Wesley and Ash (1972) reported that testosterone restored the

susceptibility of orchiectomized jirds to infection with Brugia pahangi to the level of control animals.

Summary

Testosterone treated orchiectomized rats harbored normal Hymenolepis diminuta whereas castrated controls had stunted worms. Testosterone brought back to normal the egg-output of this tapeworm in castrated rats, and increased that of Diphyllobothrium sebago in castrated male hamsters. Testosterone increased the numbers of larval Taenia taeniaeformis in castrated male rats and activated the growth of Echinococcus multilocularis cysts in castrated mice of both sexes. Testosterone increased the numbers of both larval and adult Nematospiroides dubius in orchiectomized rats. It increased the numbers of Nippostrongylus brasiliensis in castrated hamsters of both sexes and those of Trichinella spiralis in ovaricetomized mice. It restored the susceptibility of orchiectomized jirds to Brugia pahangi to that of controls.

Testosterone decreased the numbers of female Schistosoma mansoni in castrated mice. It had no effect on Amplicaecum robertsi infections in castrated mice of both sexes or on the number of Strongyloides ratti in castrated rats.

Oestrogens increased the growth of cysts of E. multilocularis in castrated mice, and the numbers of adult N. dubius in castrated female rats. Yet they did not abolish the stunting of H. diminuta in castrated rats. Oestrogens depressed the numbers of T. taeniae-

formis larvae in orchiectomized rats and those of N. dubius larvae which penetrated the intestinal wall in ovariectomized rats. Oestrogens depressed the numbers of T. spiralis larvae in castrated male mice, but had no effect on the numbers of Strongyloides ratti in castrated rats of both sexes.

In general, testosterone tends to increase the number of parasites in castrated animals to that of controls, whereas oestrogens mostly depress this number.

SPLENECTOMY AND HELMINTHIC INFECTIONS

Trematode infections

Splenectomy in mice and rats does not effect the course of infection with Schistosoma mansoni (Thompson, 1954). But splenectomized monkeys infected with S. mansoni pass more eggs and for a longer period of time than the intact controls (Bruce et al.1966).

Cestode infections

Splenectomy enhanced slightly the infections with Hydatigera taeniaeformis in mice (Hoeppli, 1941). It had no effect on the growth of larval Echinococcus multilocularis (Lee, 1970) and on that of adult Hymenolepis nana in mice (Larsh, 1944; Weinmann, 1968).

Nematode infections

Bittner (1913) showed that splenectomy had no effect on the course of experimental trichinellosis in rabbits. Negative results were obtained with Nippostrongylus brasiliensis in rats (Ogilvie and Jones, 1967), Aspicularis tetraptera in mice (Mathies, 1962) and Brugia malayi and B. pahangi in guinae pigs, mice and cats (Nooruddin and Ahmed, 1967; Burren, 1972). However, splenectomy increased the numbers of microfilariae of Loa loa in monkeys (Duke, 1960) and of those of Litomosoides carinii in cotton rats (Hawking, 1962). The same author (1962) reported that splenectomy caused little or no

recrudescence in dogs infected with either Dirofilaria immitis or D. repens, and in gerbils infected with Dipetalonema witei.

Summary

Splenectomized monkeys infected with Schistosoma mansoni pass more eggs and for a longer period of time than intact controls. Splenectomy increases the numbers of microfilariae of Loa loa in monkeys and of those of Litomosoides carinii in cotton rats.

Splenectomy has little effect on the experimental infections with Hydatigera taeniaeformis in mice, Trichinella spiralis larvae in rabbits, Dirofilaria immitis or D. repens in dogs, and Dipetalonema witei in gerbils.

It has no effect on the course of infections with S. mansoni Echinococcus multilocularis, and Hymenolepis nana in mice, Nippostrongylus brasiliensis in rats, Aspicularis tetraptera in mice, and Brugia malayi and B. pahangi in guinea pigs, mice and cats.

Splenectomy in mammals does not markedly change the course of helminthic infections. However, the spleen is important in the development of immunity to metazoan infections at least in some host-parasite systems.

CORTISONE AND HELMINTHIC INFECTIONS

Trematode infections

A small, but statistically significant reduction in the number of Schistosoma mansoni was found in the majority of cortisone treated mice (Weinmann and Hunter, 1960). Cortisone increased the susceptibility of rats to Paragonimus miyazakii infections, but not to those with the more pathogenic P. westermani (Tada, 1967).

Cestode infections

Cortisone impaired the acquired immunity of mice to infections with Hymenolepis nana . Massive superinfection occured when the primary adult worms were still present in the gut (Weinmann and Rothman, 1961).

Cortisone made mice of a resistant strain susceptible to Hydatigera taeniaeformis infections (Olivier, 1962). This author found that cortisone was effective only when given within the first 12 days of infection, and he correlated this phenomenon to the production of antibodies by mice within the same period. Esch (1964, 1967) found that cortisone had a similar effect on larvae of Taenia multiceps in mice. The largest number of larvae were found in mice which received cortisone between the 1st and 14th day post infection. But cortisone increased the number of larvae only in the subcutaneous tissue, but not in the central nervous system.

Cortisone accelerated the growth of Echinococcus multilocularis cysts in LDF_1 mice of both sexes (Lee, 1970). The initial period of slow growth was shorter in cortisone treated mice than in controls, 24 and 40 days, respectively. The difference in the weight of cysts between treated and control mice was greatest 44 to 57 days post infection. Infected mice developed a high leucocytosis with pronounced neutrophilia, but without lymphocytosis. Cortisone lowered the absolute numbers of both lymphocytes and monocytes, but had no effect on these of neutrophils.

Nematode infections

Cortisone suppressed the innate resistance of white rats to Litomosoides carinii infections and the antibody production by this host (Briggs, 1963). It did not increase the percentage of larvae completing migration to the lungs (Olson, 1959). Innate resistance of albino mice to Trichuris muris was suppressed by a single dose of cortisone, given at the time of infection or 1 day prior to it (Campbell, 1963). Cortisone abolished the acquired immunity of mice to Trichinella spiralis (Coker, 1955). Zaiman et al.(1962) found that cortisone lowered considerably the average survival time of mice heavily infected with T. spiralis (to 42.5 hours, as compared to 71.8 hours for controls). Cortisone suppressed the innate resistance of Chinese and golden hamsters to T. spiralis. Treated animals harbored adult worms for a longer period of time than the controls (Ritterson, 1959; Ritterson and Concannon, 1968).

Cortisone increased significantly the susceptibility of mice to infections with Aspicularis tetraptera (Mathies, 1962). Cortisone was used by Weinstein (1953) to establish a greater number of Nippostrongylus muris in rats. The resistance of guinea pigs to infections with N. brasiliensis was lowered by administration of cortisone. More than 10 times larger numbers of larvae completed the skin-lung migration in treated animals than in controls (Parker, 1961). Cortisone suppressed the tissue reaction of rats infected with larvae of Nematospiroides dubius. In treated animals the maturing larvae could penetrate through their capsules in the intestinal wall and return to the lumen (Cross, 1960).

Summary

Cortisone makes rats more susceptible to infections with Paragonimus miyazakii (but no P. westermani) Unexpectedly, it reduces the numbers of developing Schistosoma mansoni in mice - a phenomenon well worth of further studies.

Cortisone permits to superinfect mice with Hymenolepis nana, and makes mice of a resistant strain susceptible to Hydatigera taeniaeformis. It increases the percentage of developing larvae of Taenia multiceps and the growth rate of Echinococcus multilocularis cysts in mice.

Cortisone suppresses the innate resistance of rats to Litomosoides carinii, of mice to Trichuris muris, of Chinese and golden hamsters to Trichinella spiralis, and of guinea pigs to Nippostrongylus brasiliensis. It abolishes the acquired immunity of mice to

T. spiralis and suppresses the tissue reaction of rats to larvae of Nematospiroides dubius.

Thus cortisone is efficient in suppressing the immunity to at least some trematodes, cestodes and nematodes and the study of its effect on the infection of mice with tetrathyridia of Mesocestoides seems to be justified.

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