THE UNIVERSITY OF MANITOBA

# THE INFLUENCE OF SEX HORMONES ON THE GROWTH OF LARVAL *Echinococcus multilocularis* LEUCKART, 1863

by

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ABSTRACT

The effects of host castration and sex hormones on the growth of Echinococcus multilocularis cysts in rodents were studied. Preliminary experiments with SWR, SEC and $LD_1F_1$ mice and with gerbils have shown that cysts grow faster in female hosts. The growth of cysts is also age-dependent. Cysts grow fastest in 1-month-old mice and much slower in mice aged 6 - 10 months. In 18-month-old mice the growth is faster than in the middle-aged mice but still slower than in young mice.

Cysts grow faster in intact mice than in castrated mice of both sexes. The study of the growth curves of cysts in intact and castrated hosts showed that the initial period of slow growth of cysts is longer in castrated hosts.

Intact mice of both sexes treated with testosterone propionate or estradiol benzoate have heavier cyst loads than controls. In castrated males and females, testosterone and estradiol increase the cyst loads up to those in the intact controls or more. Experiments showed that estradiol in a dose half as large as that of testosterone accelerated the growth of cysts as much as testosterone did, and both hormone-treated groups had cyst loads similar to those of the intact controls.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure Page

LIST OF TABLES

# INTRODUCTION

Human alveolar hydatid disease is of world importance. Its causative agent is the larva of the cestode, Echinococcus multilocularis. The only effective means used presently in combating this disease are community sanitary measures and surgery. The absence of chemotherapy plus the seriousness of surgical treatment increase the importance of echinococcosis. The development of a rational chemotherapy, therefore, is a necessity.

Studies of the factors affecting the growth of cysts in mice are necessary since these hosts are used in screening possible chemotherapeutic agents. Such studies also broaden the knowledge of the biochemistry, physiology and host-parasite relationships of the causative organism which is necessary for developing a treatment for echinococcosis (Agosin 1968).

The present study examines one aspect of host-parasite interaction, namely, the influence of host sex on the growth of larval E. multilocularis. Observations made by Lubinsky (1967) indicated that the growth of its cysts is faster in female hosts. The present project was to study the influence of sex hormones on the growth of cysts. Two approaches were used.

1. Study of the effects of host castration on cyst size.
2. Study of the effects of sex hormones on the weight of cysts in intact and castrated hosts.

In preliminary experiments, the growth of cysts in both sexes of SWR and SEC mice and gerbils was studied to determine if these hosts exhibited sex resistance. Cyst growth in mice of different ages was also studied to establish whether E. multilocularis infections were subject to age resistance. Hopefully these data will aid in developing a chemotherapy of hydatid disease.

# REVIEW OF LITERATURE

## *Echinococcus multilocularis* Leuckart, 1863

### Life Cycle

A canine-rodent life cycle is typical of *Echinococcus multilocularis*. Transmission is based on the predator-prey relationship between the carnivore and rodent. Man becomes an accidental intermediate host by ingesting *Echinococcus* eggs. It has been suggested that insects such as blowflies (*Phormia* sp.) and beetle grubs (*Agonum* spp. and *Harpalus* sp.) may play a secondary role as mechanical vectors transferring the onchosphere from the definitive to intermediate host (Schiller, 1954, Leiby and Nickel, 1968).

In Alaska and the Canadian Arctic the primary source of infection for foxes are microtine rodents, while it appears that the deer mouse is the principal intermediate host for this tapeworm over much of its range in the rest of North America (Leiby et al. 1970).

The natural and experimental intermediate hosts of *E. multilocularis* are listed by Smyth and Smyth (1964) and Lee (1970).

*E. multilocularis* does not occur naturally in ungulates either domestic or wild. All attempts to infect ruminants have been unsuccessfull (Cameron 1960; Rausch and Schiller 1956; Stanger and Lubinsky 1966). No natural or experimental infections in birds of prey have been reported.

The usual rodent-carnivore life cycle (Fig. 1) can be used as a source of larval *E. multilocularis*. It is hazardous, however, because the onchospheres are infectious to man. A highly susceptible host is the

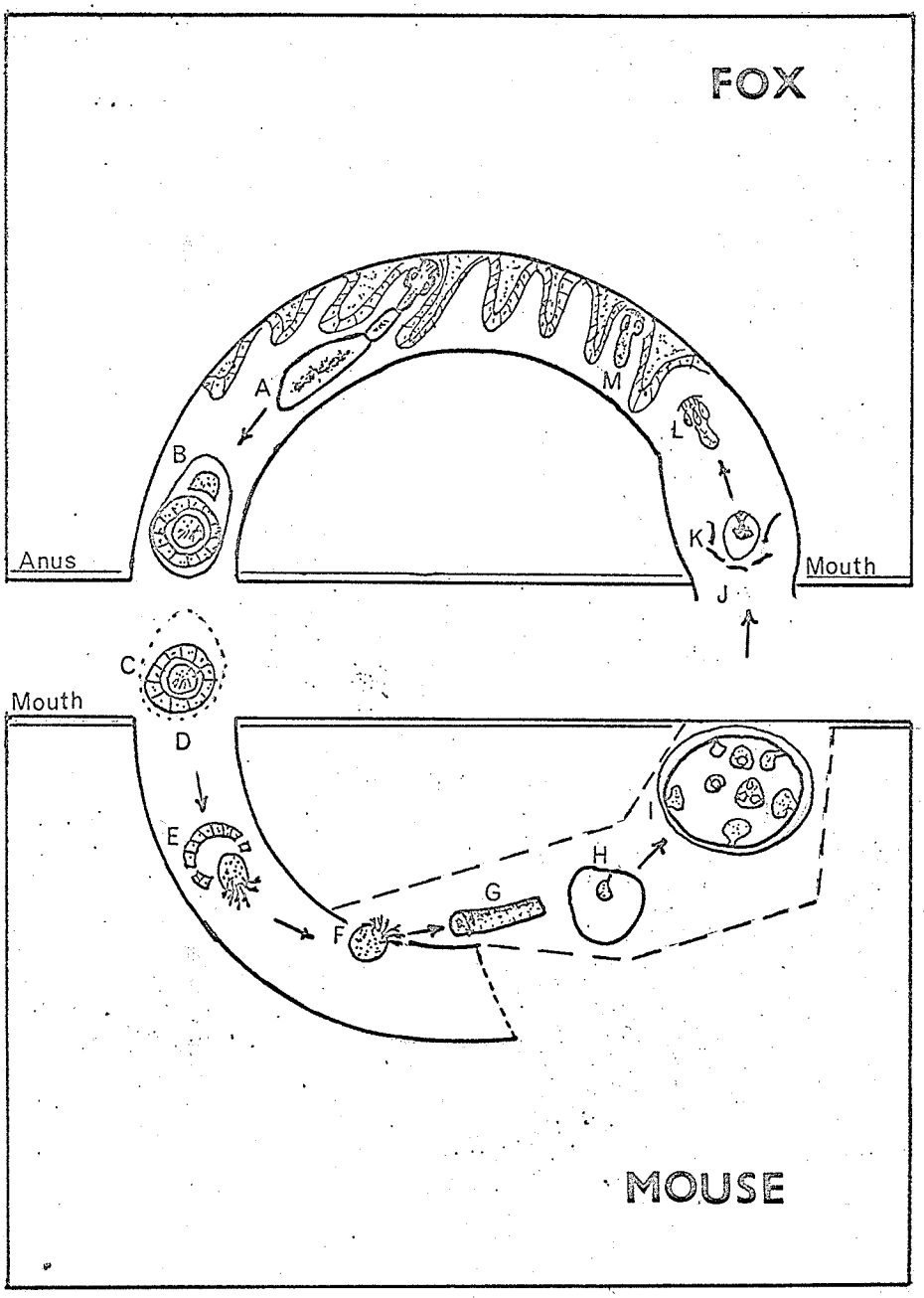



FIGURE: 1. LIFE CYCLE OF Echinococcus multilocularis

cotton rat (Sadun et al. 1957) which can be infected both primarily or secondarily. Vegetative serial transfers in which protoscoleces or acephalic cysts are parenterally transferred from cotton rat to cotton rat provide a convenient method of maintaining the parasites (Lubinsky 1960a and 1967). The growth of cysts in cotton rats is fast, over 20 gm. of cysts being produced in about 45 days (personal observation). These rats are therefore a convenient source of large quantities of infectious material necessary for experimentation with large numbers of animals.

Measurement of Larval Growth

An accurate measurement of the growth of larval E. multilocularis is difficult because this growth is often invasive. The presence of protoscoleces and their development depends on the species and strain of host rather than on growth rate of cysts.

Cyst volume and weight are the criteria presently used even though cyst clusters always contain some host tissue (Lubinsky 1960b; Lubinsky and Desser 1963).

Factors Affecting Cyst Growth

The susceptibility of hosts to infection with E. multilocularis and the growth rate of this cestode within these hosts vary greatly and are influenced by many factors. These include:

1. Host species: Rausch and Schiller (1956) were the first to show that rodents are the intermediate hosts of E. multilocularis. Sadun et al. (1957) found that cotton rats infected with eggs produce large

quantities of fertile cysts and this was confirmed by Webster and Cameron (1961) and others.

Experiments with Mus musculus have yielded conflicting results. Vogel (1957) and Mankau (1957) established infections in white mice by feeding them onchospheres,while Rausch and Schiller (1956) and Sadun et al. (1957) found them insusceptible. This brings up the possibility that the susceptibility of mice to E. multilocularis infections depends on the strain of mice.

Secondary infections of E. multilocularis have also been established in cotton rats, gerbils and white mice (Lubinsky 1960 a,b). Serial transfers in these animals result in a progressive increase in the growth rate of the cysts. Webster and Cameron (1961) pointed out that parenteral infections are successful in a much wider range of hosts than are primary infections. This probably depends on the avoidance of the liver reaction in the secondary method.

2. Host strain: Yamashita et al. (1963) stress the importance of strain differences of hosts in experimental infections with E. multilocularis. They showed that in five mouse strains, namely, fm. NC, SM, gpc and KK, susceptibility to primary infection varied greatly.

The growth of E. multilocularis cysts to various strains and hybrids of mice of the Jackson Laboratory was studied by Lubinsky and Desser (1963), and Lubinsky (1964 and 1967). Cyst growth was fast in C57L and $DBA_1$ miee. These strains were crossed (C57L female X $DBA_1$ male) and in the resulting $F_1$ hybrids, "$LD_1F_1$", cyst growth was fast, (Lubinsky, 1967). Susceptibility was initially high, about 90%, and is at present 100%. This is why these hybrids were used in the present study.

3. Host age: Schwabe et al. (1959) found that resistance to infection with onchospheres of E. granulosus in white mice increases with age. Mice 48 days old or younger were very susceptible while those 71 days or older were relatively resistant.

4. Nature and dose of infectious material: The presence of protoscoleces in the inoculum is not important in serial transfers of E. multilocularis. Acephalic "sterile" cysts can be serially transferred both in mice and in gerbils (Lubinsky, 1967), In both these hosts scoleces appear only after 3 or more months of growth. However, if such acephalic cysts are injected into cotton rats, the scoleces develop within about 20 days (Lubinsky 1969). Generally, the larger the inoculum, the faster the growth of cysts. Also, the larger the dose, the shorter the period between inoculation and the development of palpable cysts.

5. Administration route: The intraperitoneal route is the best for inoculation. In most mice, including $LD_1F_1$ and $DBA_1$ the intraperitoneal cysts grow faster than the subcutaneous ones. An exception to this is A/J mice (Lubinsky 1967).

## Influence of Gonadal Hormones on Parasitic Infections

### Sex of the Host and Helminthic Infections

The sex of the host affects both its susceptibility to certain parasites and the growth of the parasites within the host. Helminthic infections which are influenced by host sex are discussed in the following review.

Trematode infections. Male Rana temporaria have higher parasite loads than the females during the breeding season (Lees and Bass 1960). This difference becomes less evident after the breeding season. The trematodes involved are Polystoma intergerrimum and Gorgoderina vitelliloba.

Leigh (1960) showed that female gar are more heavily infected than males with metacercariae of Odhneriotrema sp. but he gave no information on the age of the hosts or the state of their gonads.

Acanthocephalan infections. Lees and Bass (1960) reported that male frogs had more Acanthocephalus ranae than the females during the breeding season but this difference was less evident after breeding season.

Adult cestode infections. The growth of adult Hymenolepis diminuta is normal in male rats on a vitamin G deficient diet, but stunted in females on the same diet (Addis 1946).

Larval cyclophyllidean infections. Campbell (1939) found that the susceptibility to infection of experimentally infected female rats to Taenia taeniaeformis was only 60 - 80 per cent that of the males.

Berg and Beck (1968) observed that natural infections with T. pisiformis occur only in 55.26 per cent of the females whereas all males were infected. Vande Vusse (1969) points out that there is no such difference and suggests that the results of Berg and Beck depend on inadequate sample size. Sex differences in the susceptibility of KK and NC mice to infection with onchospheres of Echinococcus multilocularis were demonstrated by Ohbayashi and Sakamoto (1966). The females of both strains were more resistant to infection than the males. Female mice harboured fewer and smaller cysts of E. granulosus than males when infected with scoleces (Frayha et al. 1971).

More larvae of Taenia crassiceps survive and continue dividing asexually in a female mouse than in the male (Freeman 1964). The development of larval Hymenolepis diminuta in Tribolium confusum and T. castaneum is also faster in the female host (Soltice et al. 1971) E. multilocularis cysts grow faster in female A/J, C57L and $DBA_1$ mice and $LD_1F_1$ hybrids than in male mice (Lubinsky 1967). This is also the case in Multiceps multiceps infections in mice (Esch 1967).

Nematode infections. Dobson (1965, 1966a) showed that third stage larvae of Amplicaecum robertsi are affected by host sex. No difference occurred in mice younger than 90 days when autopsied 14 DPI. In those over 90 days of age fewer larvae were recovered from the females than from males. The larvae were shorter in female mice of this age. In experimental infections of Aspicularis tetreptera in mice (Mathies 1954, 1959b), males harbour twice as many worms as the females. Thomas (1964) found that 2 year old male trout are more heavily infected with Dacnitis truttae than females.

Naturally infected male Rana temporaria have more Rhabdias bufonis than females during the breeding season (Lees and Bass 1960). Miller (1965) found that adult bitches are significantly less susceptible to infection with third stage larvae of Ancylostoma caninum than adult males.

Dobson (1961a) found that Nematospiroides dubius grows faster in male mice than in females. Male and female worms from female mice are shorter than those from males in 5 and 10 day infections (Dobson 1966b). The hamster exhibits both a difference in susceptibility between the sexes and a difference in worm burden in the case of Nippostrongylus muris. Haley (1954, 1958) showed that the incidence of experimental infections in male hamsters was 100 per cent as compared to 72 - 79 per cent in females. The mean worm burden of the males was nearly 23 times greater than that of the females. The magnitude of these differences did not vary significantly at different intervals after infection.

Nippostrongylus brasiliensis exhibits little growth difference in rats of different sex, but mature male hamsters are significantly more susceptible to infection than females (Haley 1966). In hamsters 2 to 3 weeks old, worm burdens were large, but there was no apparent sex difference. As the hosts matured the susceptibility of the females decreased faster than that of the males. Similar observations were made on the white mouse (Neafie and Haley 1962).

Male lambs are more susceptible to infections with Oesophagostomum columbianum than females (Dobson 1964). They also harbour more and larger worms than the females.

Female Rana temporaria had more Cosmocerca ornata, Aplectana acuminata and Oswaldocruzia filiformis than male frogs (Lees 1962). Dudzinski and Mykytowycz (1963) found that the number of helminths in Trichostrongylus retortaeformis infections in rabbits was related to the sex of the host. Such a correlation was not observed in Graphidium strigosum infections. In the case of T. retortaeformis the younger male rabbits were parasitized to a lesser extent than the female rabbits of the same age. Dunsmore and Dudzinski (1968) found that both these nematodes were present in larger numbers in the female rabbits. Whitlock (1937) reported that Syngamus trachea were more prevalent in naturally infected female partridges than in males. This was confirmed by Clapham (1939) who also showed that the intensity in experimentally infected chickens did not differ between the sexes, but the females succumbed to the infection earlier than the males.

Nematospiroides dubius infections in the rat, an abnormal host, are subject to sex resistance. The development is slower in males; females are initially more favourable to the parasite but after 10 days of infection no difference is evident (Dobson 1961b). Longer male and female worms were found in female hosts after 5 and 10 days of infection (Dobson 1966b). In review, the helminths which exhibit sex resistance are listed in Table I.

TABLE I

Helminths Exhibiting a Natural or Experimental Difference in Growth Between the Sexes

| | | | |
|---|---|---|---|
| TREMATODA | | | |
| Monogenea | Polystoma intergerrimum | Lees & Bass 1960 | male frogs more heavily infected |
| Digenea | Gorgoderina vitelliloba | Lees & Bass 1960 | male frogs more heavily infected |
| | Odhneriotrema sp. | Leigh 1960 | female pike more heavily infected |
| ACANTHOCEPHALA | | | |
| | Acanthocephalus ranae | Lees & Bass 1960 | male frogs more heavily infected |
| CESTODA | | | |
| Cyclophyllidea | Taenia taeniaeformis | Campbell 1939 | male rats more heavily infected with larvae |
| | T. pisiformis | Berg & Beck 1968 | male rabbits more susceptible to infection with cysticerci |
| | T. pisiformis | Vande Vusse 1970 | no sex difference |
| | T. crassiceps | Freeman 1964 | female mice more heavily infected with larvae |
| | Echinococcus multilocularis | Ohbayashi & Sakamoto 1966 | male mice more susceptible to infection with larvae |
| | E. multilocularis | Lubinsky 1967 | larvae grow faster in female mice |
| | E. granulosus | Frayha et al. 1971 | larvae grow faster in male mice |
| | Hymenolepis diminuta | Addis 1946 | adult worms grow better in male rats |
| | H. diminuta | Soltice et al. 1971 | more larvae survive in female beetles |
| | Multiceps multiceps | Esch 1967 | larvae grow faster in female mice |
| NEMATODA | | | |
| Ascaroidea | Amplicaecum robertsi | Dobson 1965, 1966a | male mice more heavily infected and larvae longer in males |

TABLE I Continued

| | | | |
|---|---|---|---|
| Oxyuroidea | Aplectana acuminata | Lees 1962 | female frogs more heavily infected |
| | Aspicularis tetraptera | Mathies 1954, 1959b | male mice more heavily infected |
| | Cosmocerca ornata | Lees 1962 | female frogs more heavily infected |
| Spiruroidea | Dacnitis truttae | Thomas 1964 | male trout more heavily infected |
| Rhabdiasoidea | Rhabdias bufonis | Lees & Bass 1960 | male frogs more heavily infected |
| Strongyloidea | Ancylostoma caninum | Miller 1965 | male dogs more susceptible to infection |
| | Graphidium strigosum | Dunsmore & Dudzinski 1968 | female rabbits more heavily infected |
| | Nematospiroides dubius | Dobson 1961a | male mice more heavily infected |
| | N. dubius | Dobson 1961b | no difference in rats |
| | N. dubius | Dobson 1966b | worms shorter in female mice and longer in female rats |
| | Nippostrongylus muris | Haley 1954, 1958 | male hamsters more susceptible to infection and more heavily infected |
| | N. brasiliensis | Haley 1966 | male rats more susceptible to infection |
| | Oesophagostomum columbianum | Dobson 1964 | male lambs more susceptible and have more and longer worms |
| | Synagamus trachea | Whitlock 1937<br>Clapham 1939 | female partridge more susceptible to infection |
| | Trichostrongylus retortaeformis | Dudzinski & Mykytowycz 1963<br>Dunsmore & Dudzinski 1968 | female rabbits more heavily infected |
| | Oswaldocruzia filiformis | Lees 1962 | female frogs more heavily infected |

## Castration of the Host and Helminthic Infections

Castration of the host is a useful technique of studying relationships between growth of parasites and gonadal hormones. From the literature it is apparent that this treatment may lead to a decrease in the host's susceptibility to infection and/or a decrease in the growth rate of the parasite or vice versa. The effects of castration of the host on various helminthic infections are discussed in the following review.

Trematode infections. Berg (1957) studied Schistosoma mansoni infections in male albino mice and found a highly significant decrease in the number of male schistosomes per mouse in castrated males.

Cestode infections. Addis (1946) found that castration of male rats caused stunting of Hymenolepis diminuta. This did not occur in the females. Beck (1952) observed a decline in egg output of singly established H. diminuta in castrated male rats.

Castration of either sex of Wistar rats had no effect on the length, wet and dry weight or water content of Hymenolepis diminuta (Landt and Goodchild 1962). Daugherty (1956) found that castration of the rat caused a decline in the rate of glycogen synthesis from both glucose and pyruvate in H. diminuta. Aldrich et al (1954) showed that the activity of transaminase systems of Hymenolepis declined after castration of the host. The fat deposition in these worms increased.

Nematode infections. Castration of the host affects Nematospiroides dubius infections in the rat. Dobson (1961b) found more worms in both sexes of castrated hosts than in controls 5 DPI but after 10 days only the castrated males harboured more worms than the controls. Normally the worms

are longer in the females. In castrated females the worms were unchanged, but they were longer in castrated males than in controls (Dobson 1966b).

The difference in susceptibility of lambs of different sexes to Oesophagostomum columbianum is abolished after gonadectomy (Dobson 1964). Normally males are more susceptible than females, but castration of the females increases their susceptibility and thus removes the difference. In laboratory mice infected with third stage larvae of Amplicaecum robertsi the males have heavier infections than the females and the worms are shorter in the females. Castration removes these differences (Dobson 1966a).

Solomon (1966) studied the effects of castration on worm burdens of hamsters infected with Nippostrongylus brasiliensis. In his first experiment he infected the hamsters 2 to 10 weeks after gonadectomy. When infected 2 weeks after castration, males had significantly less worms than sham-operated or normal controls. Males infected 6 to 10 weeks after castration had similar worm burdens to the controls. Immature males, gonadectomized and infected 3 to 6 weeks later, harboured significantly fewer worms than the controls.

Similar results were obtained when animals were castrated at the time of weaning. No such differences were observed in the females. Solomon also showed that gonadectomy resulted in a smaller worm burden if it was performed before but not after exposure to N. brasiliensis. Castration 1 day before infection was as effective as castration 5 days prior to infection. Gonadectomized male and female mice harboured significantly fewer Aspicularis tetraptera than the controls (Mathies 1959b). The effects of host castration on helminthic infections are summarized in Table II.

TABLE II

Helminths Affected by Castration of the Host

| | | | |
|---|---|---|---|
| TREMATODA<br>Digenea | Schistosoma mansoni | Berg 1957 | decrease in number of male worms in male mice |
| CESTODA<br>Cyclophyllidea | Hymenolepis diminuta | Addis 1946 | stunted growth in male rats |
| | H. diminuta | Beck 1952 | decrease in egg output of worms in the male rats |
| | H. diminuta | Aldrich et al. 1954 | transaminase activity of worms decreased |
| | H. diminuta | Daugherty 1956 | glycogen synthesis of worms decreased |
| | H. diminuta | Landt & Goodchild 1962 | no effect on length, wet and dry weight or water content |
| NEMATODA<br>Ascaroidea | Amplicaecum robertsi | Dobson 1966a | increases susceptibility of female mice |
| Strongyloida | Nematospiroides dubius | Dobson 1961b | male mice and rats more heavily infected |
| | N. dubius | Dobson 1966b | worms longer in castrated males than controls |
| | Nippostrongylus brasiliensis | Solomon 1966 | lower burdens in male hamsters |
| | Oesophagostomum columbianum | Dobson 1964 | increases susceptibility of female lambs |

Hormone Treatment and Helminthic Infections

The influence of gonadal hormones on helminthic infections has been studied by many authors.

Trematode infections. The numbers of Polystoma intergerrimum and Gorgoderina vitelliloba found in Rana temporaria can be depressed by injection of estradiol benzoate (Lees and Bass 1960).

Berg (1957) came to the conclusion that testosterone decreased the number of female Schistosoma mansoni collected from castrated males. These results were refuted by Robinson (1959) who could not find any difference in survival rates of either sex of schistosomes as a result of testosterone treatment. He believed that, Berg's results were based on too small samples.

Acanthocephalan infections. The number of Acanthocephalus ranae found in Rana temporaria can be decreased by injection of estradiol benzoate (Lees and Bass 1960). This result as well as those with trematodes are questionable since the frogs were naturally infected.

Cestode infections. Campbell (1939) and Campbell and Melcher (1940) found that the experimentally infected females harbour 33 per cent less Taenia taeniaeformis cysticerci than the males, and attempted to alter this sex difference by injections of theelin (an estrogen) and testosterone propionate. In females which received theelin the parasite load was the same as that in the controls. The males which received theelin had less cysticerci than controls. Females which received testosterone propionate showed a marked decrease in resistance as compared to controls. This was not the case in males which received testosterone.

Addis (1946) showed that castrated male rats given testosterone

parenterally or methyl testosterone orally harboured, normal Hymenolepis diminuta, in contrast to castrated controls which had stunted worms. Castrated males treated with progesterone also had normal worms. Treatment with theelin did not abolish the stunting of the worms. Neither testosterone, theelin nor progesterone offset the effects of castration in the female. Addis concluded that H. diminuta is dependent on testosterone or progesterone for normal growth.

Beck (1952) working with singly-established H. diminuta showed that a drop in egg output caused by castration of the rat could be alleviated by testosterone. Beck concluded that testosterone treatment of castrated animals stimulated both the growth of the worms and their reproductive processes. He also found that progesterone was as effective as testosterone.

Testosterone increased the egg output in H. diminuta in castrated females, but progesterone had no such effect (Beck 1952). Chorionic gonadotrophin given to rats on a vitamin deficient diet restored the egg output to normal, but castrated animals were unaffected by this treatment. Meyer and Valleau (1967) showed that testosterone given to intact or castrated male hamsters infected with Diphyllobothrium sebago increases the egg output of these cestodes.

Nematode infections. The numbers of Rhabdias bufonis found in Rana temporaria can be decreased by injection of estradiol benzoate (Lees and Bass 1960). This result is questionable because the frogs were naturally infected.

The female sex hormone influences the worm burdens of young chickens infected with Ascaridia galli. Ackert and Dewhirst (1950) administered

diethylstilbestrol to young pullets infected with this nematode. The worm counts in treated birds were lower than those in the controls. Methyl testosterone given orally to chicks had no effect on worm counts but the size of the worms was significantly greater in treated animals than in the controls (Todd and Crowdus 1951).

Sadun (1951) studied the effect of sex hormones on the number and size of A. galli in experimentally infected chicks. Twenty-one day old males received testosterone, and the females, estradiol benzoate. Both sexes receiving homologous hormones harboured smaller numbers of worms than the controls. The mean length of worms from males treated with testosterone was greater than of those from controls, but worms in female chicks treated with estradiol were smaller than those in the controls.

Sadun also found that treated chicks, necropsied 18 to 32 days after infection, harboured fewer worms than the controls. Finally he showed that worms in male chicks grow somewhat faster in hormone-treated hosts, especially during the first 10 to 12 days, the difference subsequently becoming less or disappearing completely. On the other hand, the worms in hormone-treated females grow slightly slower than in the controls.

Mathies (1959b) found that testosterone had no effect on worm burdens in either male or female mice infected with Aspicularis tetraptera. However, males given beta-estradiol had significantly lower worm burdens than the controls.

In studying the effects of castration and hormone replacement in rats infected with Nematospiroides dubius, Dobson (1961b) found that testosterone did not decrease the worm burden of castrated males.

Estradiol increased the worm burdens of castrated females as compared to that of normal females. Dobson also found that in 10 day old infections of castrated males, testosterone increased both the numbers of larvae penetrating into the gut wall and of those surviving to the adult stage. Estradiol decreased the numbers of larvae which penetrated the intestinal wall in the ovariectomized females. Finally, he showed that estradiol increased the resistance of the castrated males as compared to that of the controls. Testosterone given to castrated mice of both sexes had no effect on Amplicaecum robertsi infections (Dobson 1966a). Progesterone had no effect in either intact or castrated mice.

Testosterone propionate in doses of 5, 10 or 20 mg. shortly before infection with Nippostrongylus brasiliensis reversed the effects of gonadectomy in mature male hamsters (Solomon 1966). This hormone increased the susceptibility of normal male and normal and gonadectomized female hamsters. Progesterone was as effective as testosterone in increasing the susceptibility of normal females, whereas estradiol benzoate and cholesterol had no effect.

Stilbestrol pellets implanted into 6 to 8 week old rabbits have no effect on Trichostrongylus axei or T. colubriformis infections (Rohrbacher 1958 and 1960).

The effects of sex hormones on helminthic infections are summarized in Table III.

TABLE III

Parasites Affected by Administration of Sex Hormones

| | | | |
|---|---|---|---|
| TREMATODA | | | |
| Monogenea | Polystoma intergerrimum | Lees & Bass 1960 | estrogen decreases parasite load of frogs |
| Digenea | Gorgoderina vitelliloba | Lees & Bass 1960 | estrogen decreases parasite load of frogs |
| | Schistosoma mansoni | Berg 1957 | testosterone decreases number of female worms in castrated males |
| | S. mansoni | Robinson 1959 | testosterone had no effect in castrated males. |
| ACANTHOCEPHALA | | | |
| | Acanthocephalus ranae | Lees & Bass 1960 | estrogen decreases parasite load of frogs |
| CESTODA | | | |
| Cyclophyllidea | Taenia taeniaeformis | Campbell 1939<br>Campbell & Melcher 1940 | estrogen decreases number of larvae in male rats but not in the females<br>testosterone increases number of larvae in female rats but not in the males |
| | Hymenolepis diminuta | Addis 1946<br>Beck 1952 | testosterone and progesterone return growth and egg output of worms to normal in castrated male rats, testosterone raises egg output in castrated female rats and chorionic gonadotrophin is effective in intact males and females |
| Pseudophyllidea | Diphyllobothrium sebago | Meyer & Valleau 1967 | testosterone increases egg output of worms in intact and castrated hamsters |
| NEMATODA | | | |
| Ascaroidea | Ascaridia galli | Ackert & Dewhirst | diethylstilbestrol decreases worm counts of chickens |
| | A. galli | Todd & Crowdus 1951 | testosterone increases size of worms in chicks |

TABLE III Continued

| | | | |
|---|---|---|---|
| | A. galli | Sadun 1951 | homologous hormone decreases number of worms in both sexes of chickens. testosterone increases length of worms in male chickens. estrogen decreases their length in female chickens |
| | Amplicaecum robertsi | Dobson 1966a | testosterone and progesterone had no effect on larvae in mice |
| Strongyloidea | Nematospiroides dubius | Dobson 1961b, 1966b | estrogen decreases susceptibility of female rats, testosterone increases susceptibility of male rats |
| | Nippostrongylus brasiliensis | Solomon 1966 | testosterone and progesterone increase susceptibility of castrate and intact male and female hamsters. estrogen and cholesterol were ineffective |
| | Trichostrongylus axei | Rohrbacher 1958, 1960 | stilbestrol had no effects in rabbits |
| | T. colubriformis | Rohrbacher 1958, 1960 | stilbestrol had no effects in rabbits |
| Oxyuroidea | Aspicularis tetraptera | Mathies 1959b | estrogen lowers burdens in male mice |
| Rhabdiasoidea | Rhabdias bufonis | Lees & Bass 1960 | estrogen lowers worm burdens of frogs |

## MATERIALS AND METHODS

### Parasite and Hosts

The strain of *Echinococcus multilocularis* used in the present study was derived from adults found in foxes by Rausch and Schiller (1951) on St. Lawrence Island, Alaska, in 1950. Dr. Lubinsky, using cotton rats infected with oncospheres obtained from adult cestodes of this original strain, established a vegetatively propagated strain in 1958. Fertile cysts from the 57th - 64th transfers in cotton rats were used as a source of infection for all experimental animals.

The rodents used were of the following species:

| | |
|---|---|
| Gerbil | *Meriones unguiculatus* |
| Cotton Rat | *Sigmodon hispidus* |
| Laboratory mouse | *Mus musculus* |
| Strains | SWR |
| | SEC |
| Hybrids | $LD_1F_1$ (Cross between C57L female and $DBA_1$ male) |

Cotton rats used were the progeny of wild cotton rats from Arizona, U.S.A. Laboratory mice were obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, U.S.A. Gerbils were the progeny of a strain raised in the animal holding facilities, Department of Zoology, University of Manitoba.

The cotton rats and gerbils were housed in large metal cages with wood shavings as bedding. The mice were kept in groups of 5 on wood shavings in metal cages (10" x 7 1/2" x 4 1/2" deep) with a perforated lid with built-in pellet hopper. The light cycle in the animal rooms was 15 hours simulated daylight and 9 hours darkness, the lights being turned on at 6:00 a.m. and off at 9:00 p.m. The room temperature was maintained at 70 degrees Fahrenheit (20°C) and the humidity at 50 per cent.

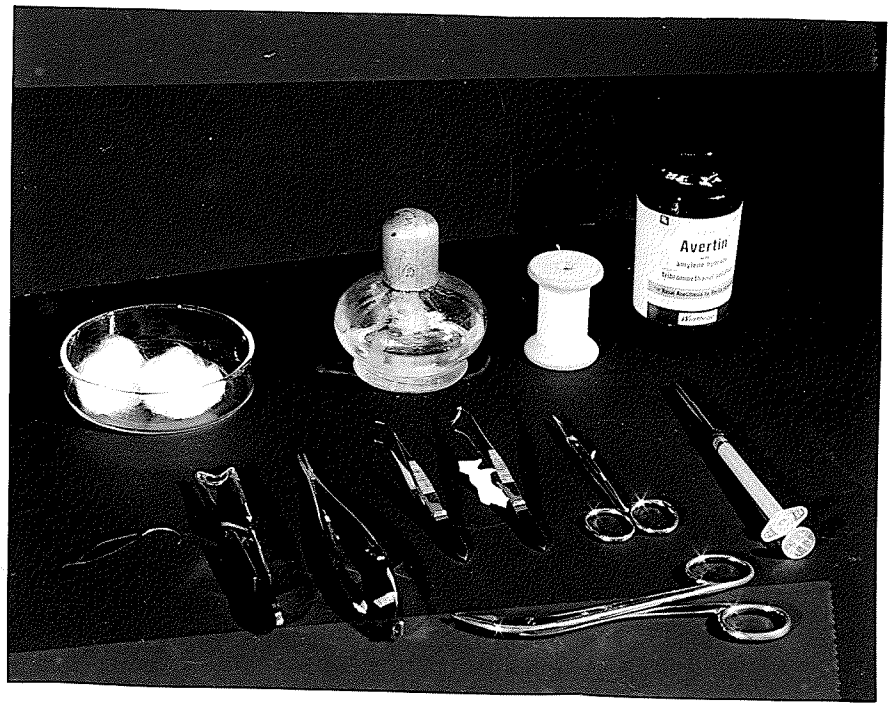
All rodents were fed Purina Lab chow and water ad libitum. Experimental animals were identified by ear notching and cages were individually labelled with experiment and group numbers.

Infection of Experimental Animals

All animals were infected with vegetatively propagated larval E. multilocularis cysts from the cotton rat transfers. The transfer technique is similar to that used for the maintenance of solid tumors by serial transfers.

Transfer rats with 50-90-day-old infections of E. multilocularis were killed with chloroform, pinned on a dissection board, shaved on the ventral side and disinfected with 70 per cent ethanol. A longitudinal cut from the sternum to the pubis was made and the viscera were exposed by reflecting the abdominal wall. Fertile cysts (containing scoleces) were removed into sterile physiological saline (0.85 per cent w/v NaCl solution) in a Petri dish. Penicillin G (Crystapen, Glaxoallenburg, Canada) was added to the saline to inhibit

Figure 2. Apparatus used for castration of male and female mice.


Avertin

bacterial growth. Cysts were removed from the rat carefully avoiding a perforation of the intestine.

Cyst clusters were ground against a sterile wire mesh (7 mesh/cm.) to yield cyst fragments containing intact protoscoleces. This material was diluted with sterile physiological saline to which penicillin G was added in concentrations of 100-1000 I.U./ml. Concentrations of 20-40 per cent cyst material were used.

Mice and gerbils were infected intraperitoneally using a 1/2", 18 gauge needle on a 3 ml syringe. The volume injected was 0.5 ml. for mice and 1.0 ml. for gerbils. By repeatedly aspirating and ejecting cyst material with a syringe it was kept in suspension, thereby some degree of uniformity in making the infections was achieved.

Castration

Mice of matched age and weight were selected for castration, placed in clean cages and allowed to settle a few days prior to surgery.

Before surgery each mouse was anesthetized with ether and injected with one per cent Avertin (tribromoethanol, Winthrop) aqueous solution prewarmed to 40°C, 0.01 ml. representing a dose of 0.1 mg/gm. body weight. The narcosis lasted for 30-45 minutes. The passing of anesthesia was characterized by increased abdominal and thoracic respiration, shivering and staggering around.

All surgery was carried out in a sterile room. Surgical equipment is shown in Figure 2. Operations were performed as follows.

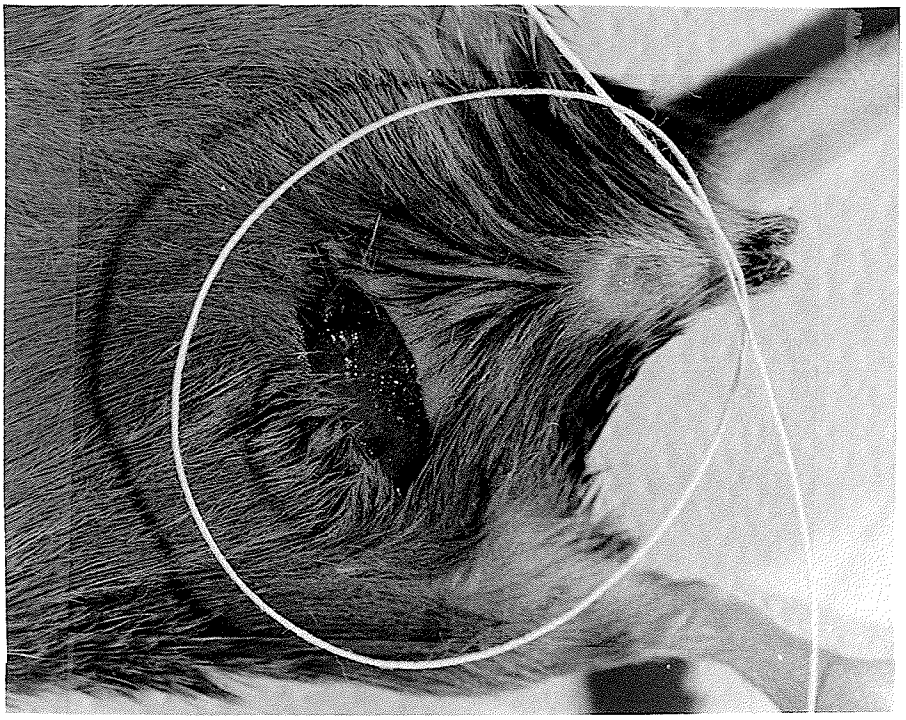
Figure 3. Castration of male mice.

A. Incision through skin and musclature;

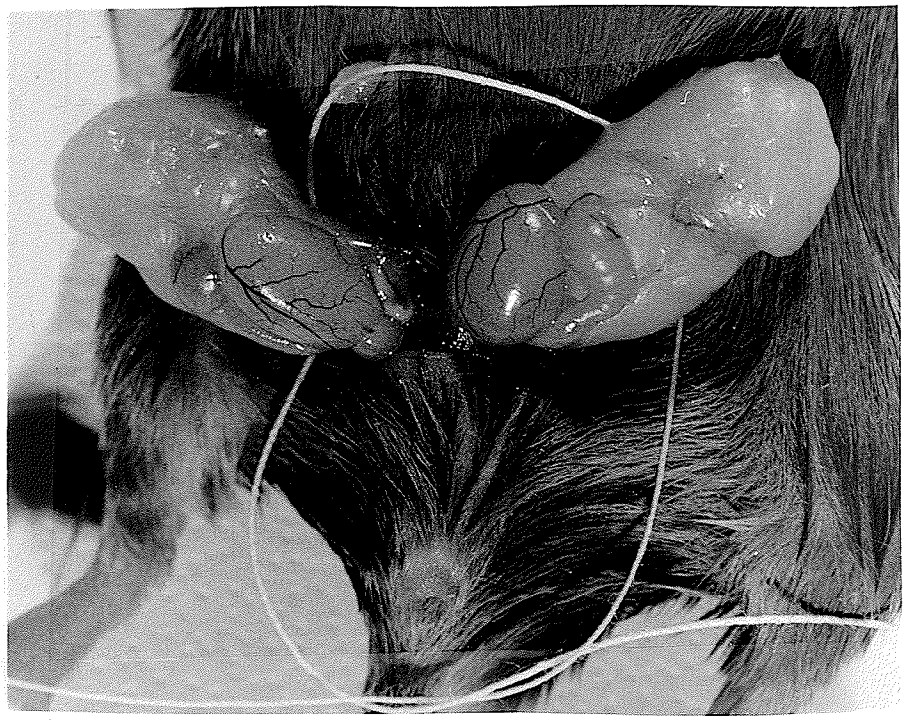
B. testes brought through the opening;

C. musclature closed with sutures;

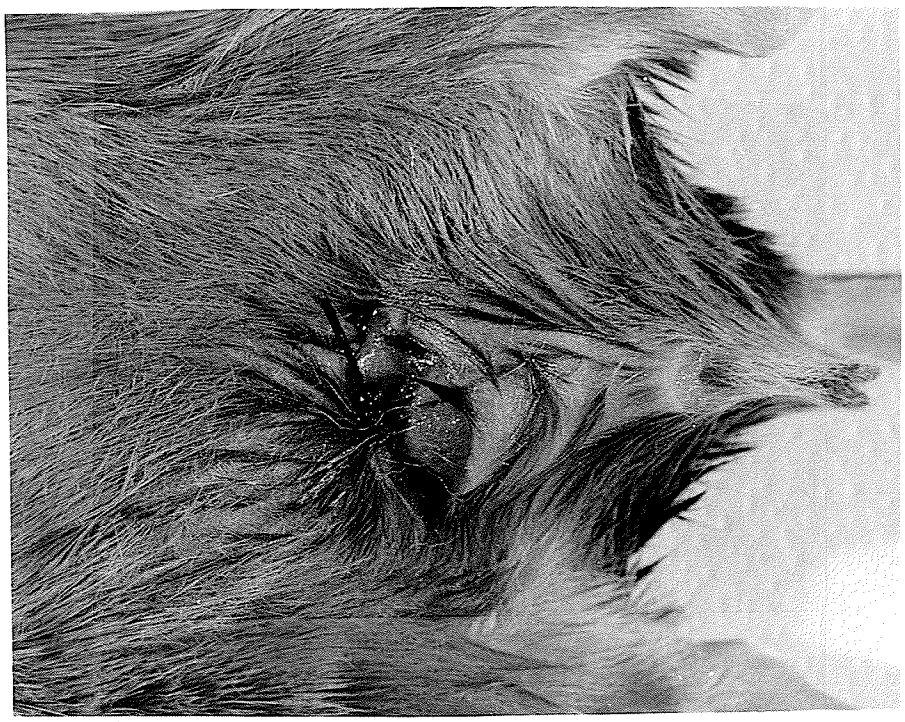
D. skin wound closed with wound clip.



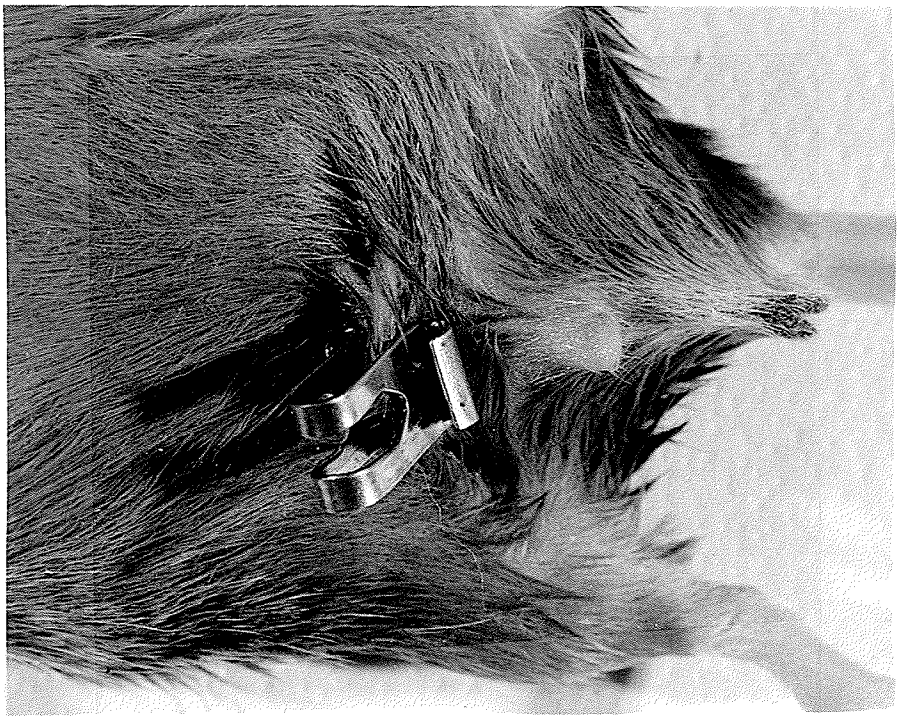
A.



B.



C.



D.

Males. The anesthetized mice were placed on their back and their ventral surface was rubbed with 70 percent ethanol. A transverse 1-cm. incision was made through the skin approximately 1 cm. anteriorly to the penis. A somewhat smaller incision was made through the musclature. The testes were secured with forceps and pulled through the opening in the abdominal wall. The vasa deferentia were tied off with attendant blood supply and the testes and epididymides removed. The incision in the musclature was closed with 1 or 2 stitches, using a round bodied, 1/2", #18 needle and black braided surgical silk, Ethicon, 000. The incision in the skin was closed with a 9-mm. wound clip (Autoclips, Clay-Adams) (Fig. 3).

Females. Anesthetized females were placed dorsal side up and rubbed with 70 per cent ethanol. A transverse 1 cm. incision was made on the dorsal surface through the skin. This was followed by smaller incisions in the musclature on either side of the vertebral column over the area of the kidneys. The ovaries could be seen through the peritoneum as small pinkish spots. Each ovary was secured with forceps, tied off and removed. The incisions were closed in a similar manner to those in the males (Fig. 4).

In both sexes the operations could be completed within five minutes with the help of an experienced assistant. Thus animals for one experiment could be castrated on the same day.

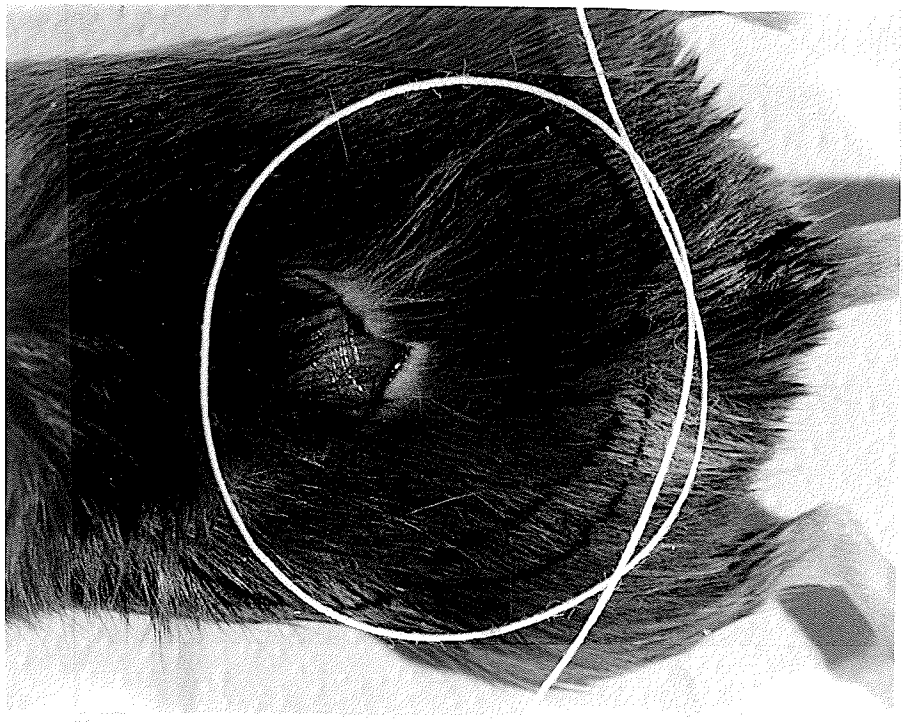
In most cases castration was performed 7 days prior to infection. This period was sufficient for the wound to heal almost completely. In one experiment the animals were operated 30 days prior to infection.

Figure 4. Castration of female mice.

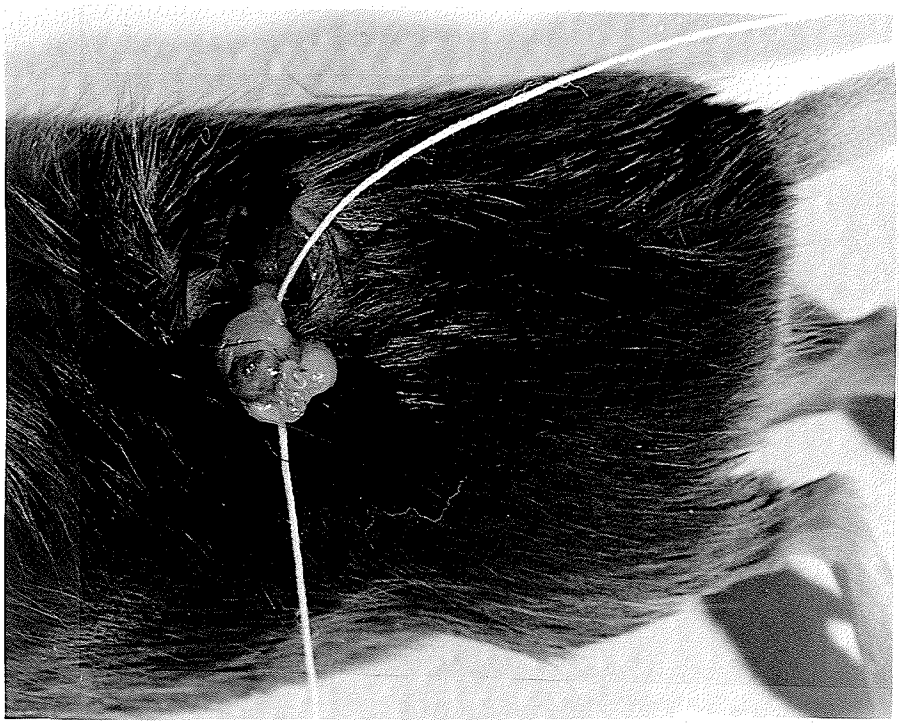
A. Incision through skin and musclature;

B. ovary brought through the opening, tied off and removed;

C. skin wound closed with wound clip.

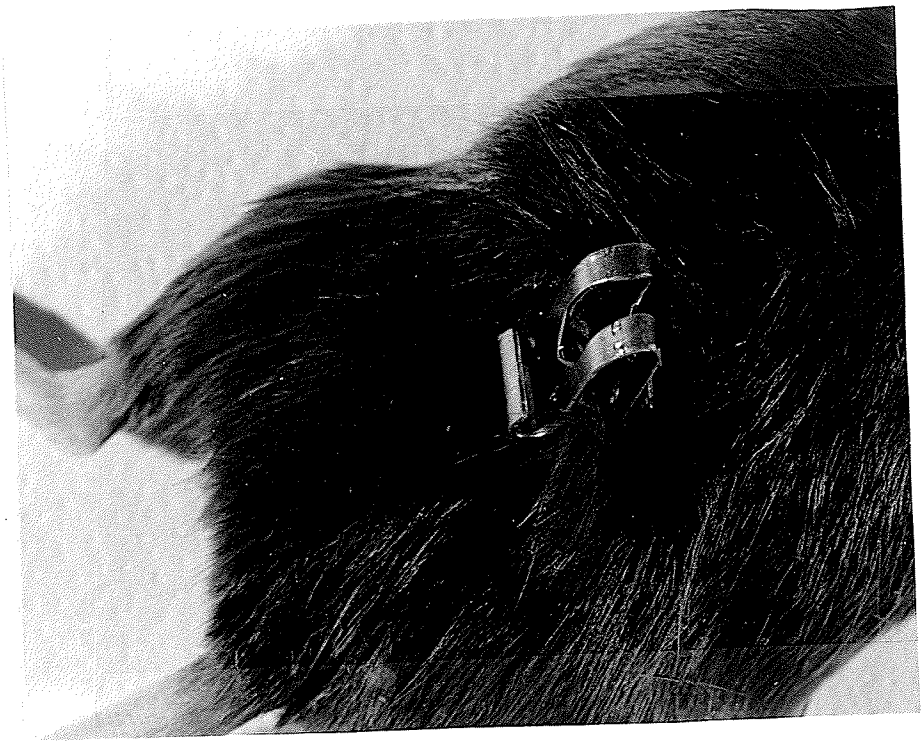


A.



B.

C.



Hormone Treatment

$LD_1F_1$ mice received sex hormone injections in several experiments. Under light ether anesthesia, each mouse received a subcutaneous injection in the lumbar region. The hormones were injected with a 20-to 24-gauge needle using a 1 ml tuberculin syringe. Testosterone propionate (Orchisterone-P, Charles E. Frosst and Co.) and estradiol benzoate (Oestroform, British Drug Houses) were given as a suspension in sesame oil. Chorionic gonadotrophin (Antuitrin-S, Parke-Davis Co.) was suspended in sterile physiological saline prior to injection.

Autopsy Procedures

The mice in each experiment were autopsied in a single day. Animals were killed with chloroform, weighed and pinned on a dissecting board. A longitudinal cut from the sternum to the pubis was made and the viscera were exposed by reflecting the abdominal wall. All cysts were removed with as little host tissue as possible. Wet weights of the cysts were determined and recorded. Wet weights of the spleen, both kidneys, both ovaries, both testes and uterus were also recorded in some experiments.

Histological Techniques

Routine histological examinations of host tissues and cysts were made for all experimental groups. Tissues were fixed and stored in ten per cent formalin. They were then washed, dehydrated and embedded in paraplast. Sections were cut at 5 to 10 microns and stained with haematoxylin and eosin. Squash preparations of unfixed cysts were made and examined under low power for protoscoleces.

Photography

A Konica camera with built in light meter and 55 mm. f/1.8 lens was used to take 35 mm. pictures. Enlargements were made on Kodak Polycontrast F paper.

Statistical Analyses

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 having no statistical significance.

## RESULTS

### Growth of Cysts in Gerbils, SEC, SWR and $LD_1F_1$ Mice

The growth of larval E. multilocularis is faster in the female $LD_1F_1$, A/J, C57L and $DBA_1$ mice (Lubinsky 1967). One objective of this preliminary study was to find whether cyst growth was influenced by host sex in other mouse strains and in gerbils. A secondary objective was to find a mouse strain in which cysts would grow as fast as in $LD_1F_1$ hybrids which were costly.

A total of 90 mice and 10 gerbils were divided into 8 groups as follows:

| | | | |
|---|---|---|---|
| Group I | - 15 ♂ SWR | Group V | - 15 ♂ $LD_1F_1$ |
| Group II | - 15 ♀ SWR | Group VI | - 15 ♀ $LD_1F_1$ |
| Group III | - 15 ♂ SEC | Group VII | - 4 ♂ gerbils |
| Group IV | - 15 ♀ SEC | Group VIII | - 6 ♀ gerbils |

All mice and gerbils were 3 months old at infection. Cyst weights were recorded at autopsy 90 DPI (days post infection).

Females always had more cysts, however, the difference was only significant in SWR mice ($P < .02$), Table IV. The difference in the cyst weights between male and female $LD_1F_1$'s was large but so was individual variability. This is reflected in the large standard error of the means. Nevertheless the highest cyst weights were observed in the $LD_1F_1$ hybrids; $2.49 \pm 0.65$ gm. in the males and $5.14 \pm 1.23$ gm. in the females. Enlargement of the spleen was observed in all animals. Values 2 and 3 times the normal

TABLE IV

Growth of Larval E. multilocularis in Different Hosts

| Host | Sex | Number Autopsied | Gross Weight at Autopsy gm. | Spleen Weight gm. | Cyst Wt. X 100 / Body Wt.- Cyst Wt. | Cyst Weight gm. | t | P |
|---|---|---|---|---|---|---|---|---|
| SWR | ♂ | 13 | 25.39 ± 0.79 | 0.15 ± 0.01 | 0.45 | 0.10 ± 0.01 | | |
| | ♀ | 13 | 25.00 ± 0.40 | 0.22 ± 0.01 | 0.88 | 0.22 ± 0.04 | 2.64 | < .02 |
| SEC | ♂ | 15 | 28.72 ± 0.45 | 0.15 ± 0.01 | 0.59 | 0.64 ± 0.19 | | |
| | ♀ | 15 | 23.80 ± 0.62 | 0.21 ± 0.03 | 0.76 | 0.75 ± 0.24 | 0.35 | > .05 |
| $LD_1F_1$ | ♂ | 15 | 31.45 ± 0.58 | 0.28 ± 0.03 | 8.6 | 2.49 ± 0.65 | | |
| | ♀ | 14 | 28.26 ± 1.54 | 0.29 ± 0.06 | 16.5 | 5.14 ± 1.23 | 1.93 | > .05 |
| Gerbils | ♂ | 4 | 81.08 ± 2.94 | 0.54 ± 0.13 | 26.6 | 17.05 ± 2.05 | | |
| | ♀ | 6 | 72.88 ± 5.47 | 0.29 ± 0.05 | 38.4 | 20.67 ± 3.98 | 0.69 | > .05 |

weight of about 0.07 gm. were observed and the enlargment was greater in females than in males. It seemed to be correlated with the cyst weight.

Because the growth of cysts was fast in $LD_1F_1$ mice it was decided to continue using these hybrids. Gerbils were not used, even though they are good experimental hosts, because large numbers of them were not available.

In the following 4 experiments the growth of cysts in intact male and female $LD_1F_1$ mice was compared. The animals used in these comparisons were in fact the intact controls of the castration experiments. These comparisons were made to confirm that cyst growth is faster in the female $LD_1F_1$ mouse.

Experiment 1. Fifteen male and 15 female mice, 2 -months-old, were autopsied 60 DPI. The mean cyst weight in the females was $4.17 \pm 0.76$ gm. and in the males $2.74 \pm 0.56$ gm. (Fig. 6). This difference was not statistically significant (Table V).

Experiment 2. The number of experimental animals was increased with the hope that statistically significant results could be obtained in this way. Consequently 20 male and 20 female $LD_1F_1$ mice, 4 months old, were injected and autopsied at 65 DPI. The difference in cyst growth between the sexes was even less than in Experiment 1, (Fig. 6) and consequently was not significant (Table V).

Experiment 3. This experiment was basically a repeat of the previous one, the only differences being that the concentration of the infectious material was slightly higher (Table V) and the mice were autopsied 69 DPI. The mean cyst weight in the males was $3.96 \pm 0.54$ gm. and in the females $6.37 \pm 0.76$ gm. (Fig. 6). This difference was highly significant ($P < .02$).

TABLE V

Growth of Larval E. multilocularis in $LD_1F_1$ Mice

| Exp. | Sex | Age at Infection Mos. | Cyst Conc. % | Number Autopsied | Autopsy DPI[a] | Cyst Weight | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | gm. | t | P |
| 1 | ♂ | 2 ½ | 33 | 15 | 60 | 2.74± 0.56 | | |
| | ♀ | 2 ½ | 33 | 13 | 60 | 4.17± 0.76 | 1.88 | > .05 |
| 2 | ♂ | 4 | 28 | 19 | 65 | 3.45± 0.54 | | |
| | ♀ | 4 | 28 | 18 | 65 | 4.30± 0.93 | 0.73 | > .05 |
| 3 | ♂ | 4 | 31 | 18 | 69 | 3.96± 0.54 | | |
| | ♀ | 4 | 31 | 19 | 69 | 6.37± 0.76 | 2.55 | < .02 |
| 4 | ♂ | 2 | 40 | 19 | 53 | 4.24± 0.57 | | |
| | ♀ | 2 | 40 | 20 | 53 | 7.34± 0.78 | 3.68 | < .001 |

[a]Days post infection

Experiment 4. In the final experiment 20 male and 20 female, 2-month-old $LD_1F_1$ mice were infected with a high concentration of cysts (40%) and autopsied 53 DPI. The difference in cyst weights was highly significant (Fig. 6). Indeed, the difference was the greatest observed (Table V).

## Age of the Host and Growth of Cysts

The possibility that host age influences the growth of cysts in the mouse was studied. This is suggested by the fact that young mice have greater cyst loads than older mice and that the greatest differences in cyst size between the male and female host occur in young mice. Therefore, the following preliminary experiment was performed.

Five groups of male $LD_1F_1$ mice, 1, 6, 10, 14 and 18 months respectively were infected with a 30 per cent suspension and autopsied 60 DPI (Table VI) (Fig. 5).

In the youngest mice, one-month-old, the growth of cysts was fastest. Growth of cysts in 6-month-old mice was significantly slower ($P < .02$). It increased slightly in the succeeding age groups, but even in the oldest mice it never reached the growth rate in the youngest.

The net weight change in one-month-old mice was over 7 gm. (Table VI). Indeed they were the only group to gain weight. All succeeding groups showed a loss in net body weight which increased with the age of mice. From this preliminary experiment it follows that Echinococcus infections are influenced by the age of the host.

## Castration

The fact that cyst growth is greater in the female host than in the male suggests that this growth is influenced by sex hormones. Removal

Figure 5. The influence of host age on the growth of Echinococcus cysts.

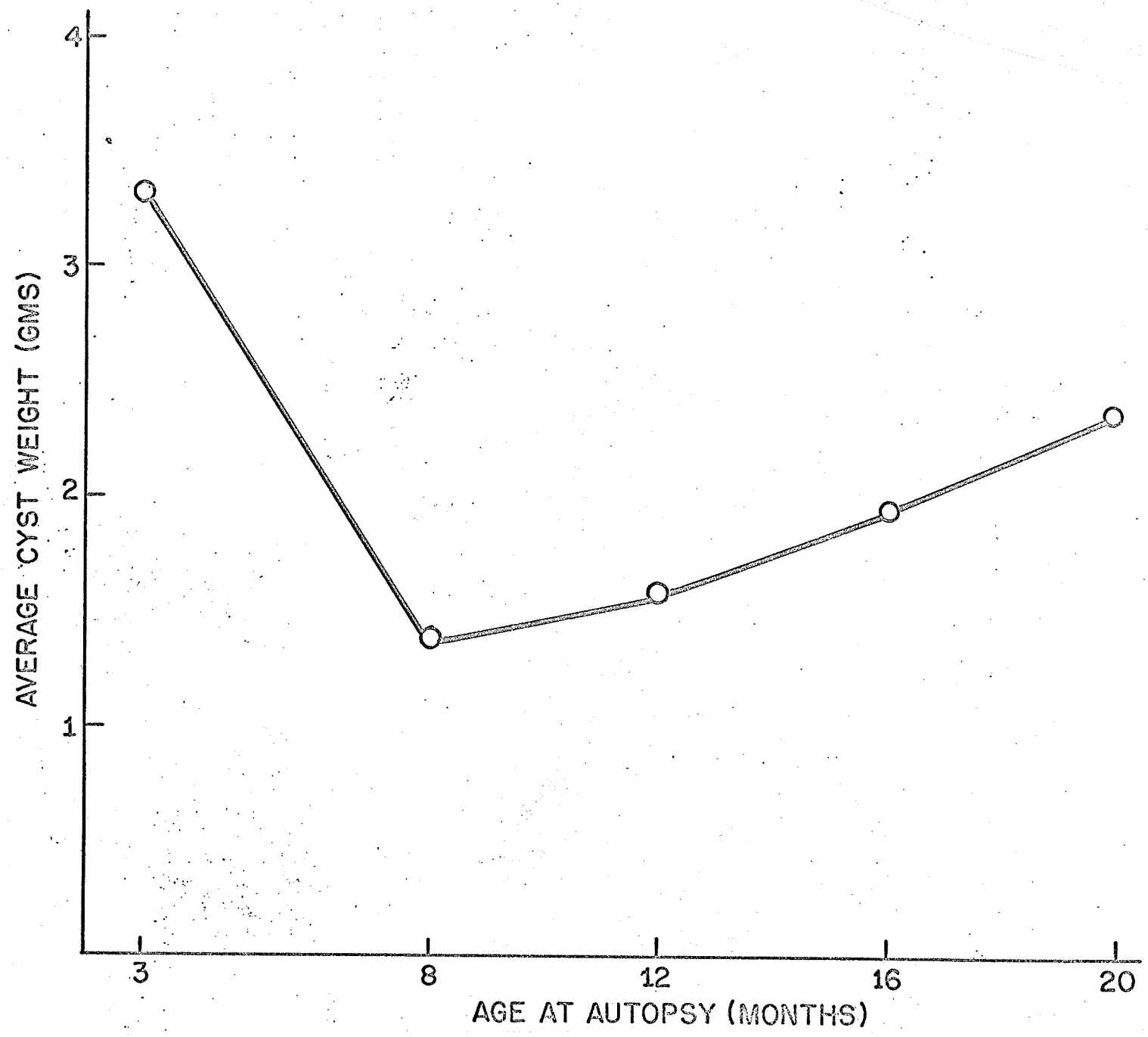

AVERAGE CYST WEIGHT (GMS)
4
3
2
1
3
8
12
16
20
AGE AT AUTOPSY (MONTHS)

TABLE VI

Growth of Larval E. multilocularis[a] in Male $LD_1F_1$ Mice

| Group | Age at Infection | Number[b] Autopsied | Weight at Infection | Net[c] Weight at Autopsy | Weight[d] Change | Cyst Weight | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mos. | | gm. | gm. | gm. | gm. | t | | P |
| I | 1 | 12 | 18.30 | 25.78 | +7.48 | $3.31 \pm 0.57$ | | | |
| II | 6 | 14 | 31.30 | 31.37 | +0.07 | $1.39 \pm 0.44$ | I vs II | 2.67 | $< .02$ |
| III | 10 | 12 | 34.60 | 34.17 | -0.43 | $1.68 \pm 0.56$ | I vs III | 2.03 | $< .05$ |
| IV | 14 | 15 | 33.20 | 31.89 | -1.31 | $2.10 \pm 0.42$ | I vs IV | 1.72 | $> .05$ |
| V | 18 | 6 | 34.70 | 32.02 | -2.68 | $2.38 \pm 0.60$ | I vs V | 1.01 | $\geq .05$ |

[a] Cyst concentration of infectious material = 30%

[b] Autopsied at 60 days post infection

[c] Net Weight = Gross weight - Cyst weight

[d] Weight change = Net weight at autopsy - Weight at infection

of the glands producing the greatest amounts of these hormones is a simple method of determining if these hormones area affecting cyst growth. Castration was therefore performed on both sexes of mice and the effects on cyst weight were studied. Three series of experiments were made. These were:

1. Preliminary experiment.
2. Protracted experiments with $LD_1F_1$ mice.
3. Comparison of cyst growth curves in castrated and intact females.

1. Preliminary Experiment. My objective was to establish whether or not castration of the host had any effects on cyst growth. Groups of 15 male and 15 female $LD_1F_1$ mice were castrated and subsequently infected. The weight of cysts in these animals was compared to that in intact mice. The results are summarized in Table VII.

Experiment 1. Sixty $LD_1F_1$ mice were used. They were divided into 4 groups.

Group I - 15 intact, infected male controls.

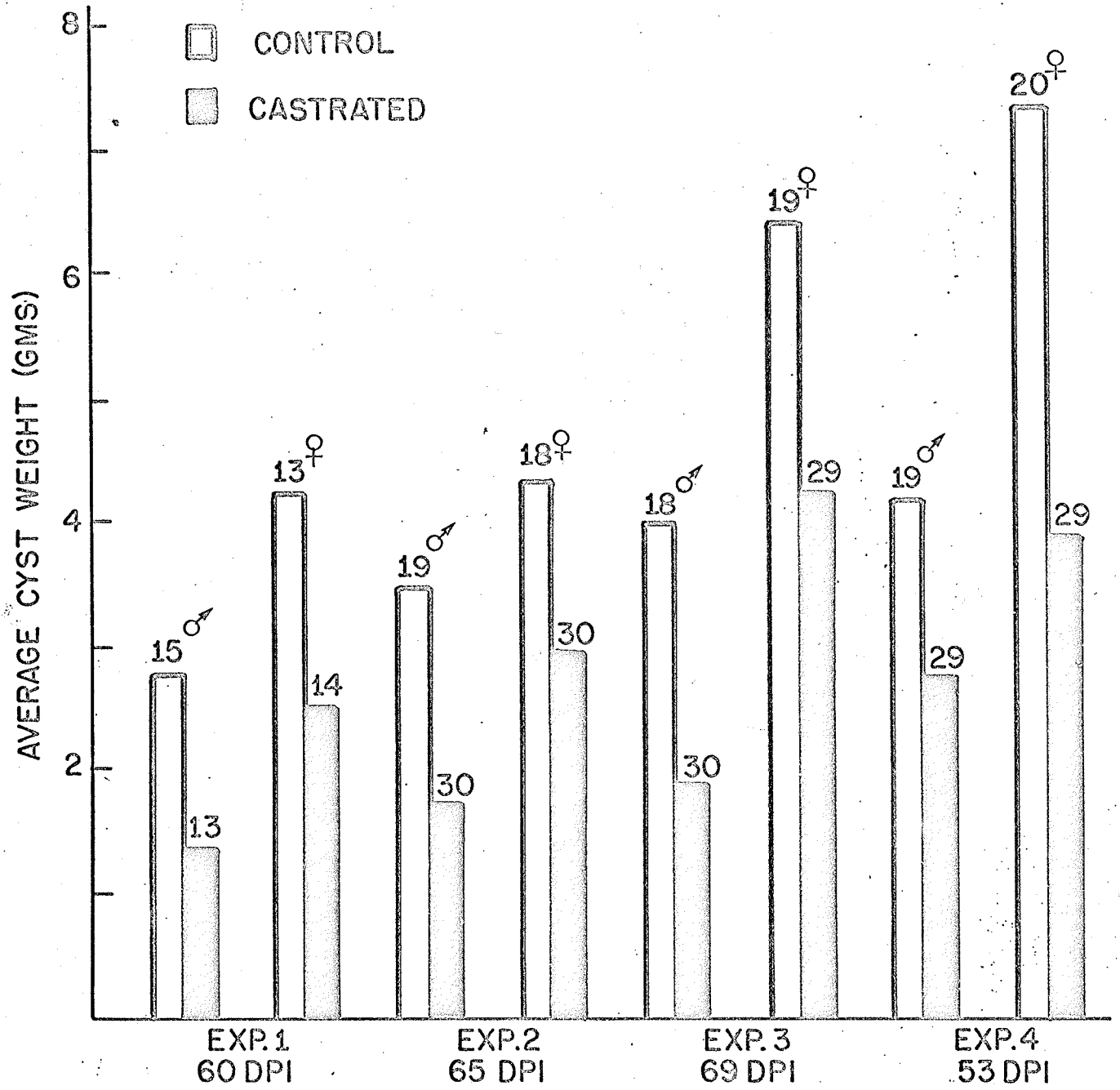
Group II - 15 castrated, infected males.

Group III - 15 intact, infected female controls.

Group IV - 15 castrated, infected females.

Castration was performed 7 days prior to infection. A suspension of cyst material having a concentration of 33 per cent was used to infect the mice which were 2½ months old at infection. Autopsy was performed 60 DPI.

The mean cyst weights of Group I and Group II; Group III and Group IV were compared. It was found that castration results in a decrease in cyst weight (Fig. 6). The cyst weight in intact males, Group I, was $2.74 \pm 0.56$ gm. while in the castrated males, Group II, it was $1.33 \pm 0.37$ gm. This difference was significant ($P < .05$). In intact females, Group III, the cyst weight was $4.17 \pm 0.76$ gm. and in castrated females, Group IV,


CONTROL
CASTRATED
AVERAGE CYST WEIGHT (GMS)
8
6
4
2
15♂
13
13♀
14
19♂
30
18♀
30
18♂
30
19♀
29
19♂
29
20♀
29
EXP.1
60 DPI
EXP.2
65 DPI
EXP.3
69 DPI
EXP.4
53 DPI

$2.46 \pm 0.64$ gm. This difference was not significant.

Histologically the cysts in all groups were similar as was the inflammatory response of the host and the distribution of the cysts within the abdominal cavity. No protoscoleces were seen. Similar observations were made in the subsequent experiments on castration and hormone treatment. Exceptions are mentioned where they occur.

An enlargement of the spleen was observed in all groups. Its weight in healthy mice was about 0.77 gm. but in mice of the experimental group spleen weight was 5 and 6 times greater, and was greater in the females than in the males and less in the castrated animals than in the controls.

2. Protracted Experiments with $LD_1F_1$ Mice. A series of 3 experiments was conducted using large numbers of male and female $LD_1F_1$ mice. They were castrated prior to infection and cyst weights in castrated and intact animals were compared. The results are summarized in Table VII.

Experiment 2 lasted 65 days. One hundred mice, 4 months of age, were divided into 4 groups,

Group I - 20 intact, infected male controls.

Group II - 30 castrated, infected males.

Group III - 20 intact, infected female controls.

Group IV - 30 castrated, infected females

Groups II and IV were operated 7 days before infection. The concentration of the cyst material in the inoculum was 29 per cent.

Cyst weights: Results similar to those of the initial experiment were obtained. The mean cyst weight in the intact controls was $3.45 \pm 0.79$ gm., that in the castrated males was $1.07 \pm 0.36$ gm. This difference was significant ($P < .02$). In intact females the mean cyst weight was $4.30 \pm 0.93$ gm. and in

TABLE VII

Growth of Larval E. multilocularis in Castrated $LD_1F_1$ Mice

| Exp. | Group | Sex | Age at Infection mos. | Castration (days before infection) | Cyst Conc. % | Number Autopsied | Autopsy DPI[a] | Cyst Weight gm. | t | P |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I control | ♂ | 2 ½ | – | 33 | 15 | 60 | 2.74± 0.56 | | |
| | II castrated | ♂ | 2 ½ | 7 | 33 | 14 | 60 | 1.33± 0.37 | 2.05 | < .05 |
| | III control | ♀ | 2 ½ | – | 33 | 13 | 60 | 4.17± 0.76 | | |
| | IV castrated | ♀ | 2 ½ | 7 | 33 | 14 | 60 | 2.46± 0.64 | 1.74 | > .05 |
| 2 | I control | ♂ | 4 | – | 28 | 19 | 65 | 3.45± 0.72 | | |
| | II castrated | ♂ | 4 | 7 | 28 | 30 | 65 | 1.07± 0.36 | 2.44 | < .02 |
| | III control | ♀ | 4 | – | 28 | 18 | 65 | 4.30± 0.93 | | |
| | IV castrated | ♀ | 4 | 7 | 28 | 30 | 65 | 2.87± 0.43 | 1.55 | > .05 |
| 3 | I control | ♂ | 4 | – | 31 | 18 | 69 | 3.96± 0.54 | | |
| | II castrated | ♂ | 4 | 7 | 31 | 30 | 69 | 1.85± 0.27 | 3.88 | < .001 |
| | III control | ♀ | 4 | – | 31 | 19 | 69 | 6.37± 0.76 | | |
| | IV castrated | ♀ | 4 | 7 | 31 | 29 | 69 | 4.15± 0.59 | 2.33 | < .05 |
| 4 | I control | ♂ | 2 | – | 40 | 19 | 53 | 4.24± 0.57 | | |
| | II castrated | ♂ | 2 | 30 | 40 | 29 | 53 | 2.76± 0.41 | 2.15 | < .05 |
| | III control | ♀ | 2 | – | 40 | 20 | 53 | 7.34± 0.78 | | |
| | IV castrated | ♀ | 2 | 30 | 40 | 29 | 53 | 3.88± 0.33 | 4.57 | < .001 |

[a] Days post infection

castrated females 2.87$\pm$ 0.43 gm. (Fig. 6). This difference was not statistically significant.

Host reactions: (Table VIII). A total of 3 mice, 1 from Group I and 2 from Group III died before termination of the experiment. No mice were lost from either of the castrated groups. It was expected that mortality would be higher in the controls since cyst growth in these is faster than in the castrated mice.

Spleen enlargment was greater in female than in male controls, and greater in castrated females than in castrated males.

The gross body weights in all 4 groups increased (Fig. 7). Group I and Group III gained 4.0 gm. and 5.0 gm. respectively. On the other hand the net body weight (gross wt. minus cyst wt.) in both groups increased only by 0.75 gm. In Group II there was an initial increase in gross weight followed by a slight decrease and levelling off at about 30 DPI. The net body weight actually decreased 0.14 gm. during this experiment. In Group IV gross body weight increased substantially while the net body weight increased by 1.10 gm.

Experiment 3. This experiment was a replication of the previous one. A total of 100, 4-month-old $LD_1F_1$ mice were divided into 4 groups.

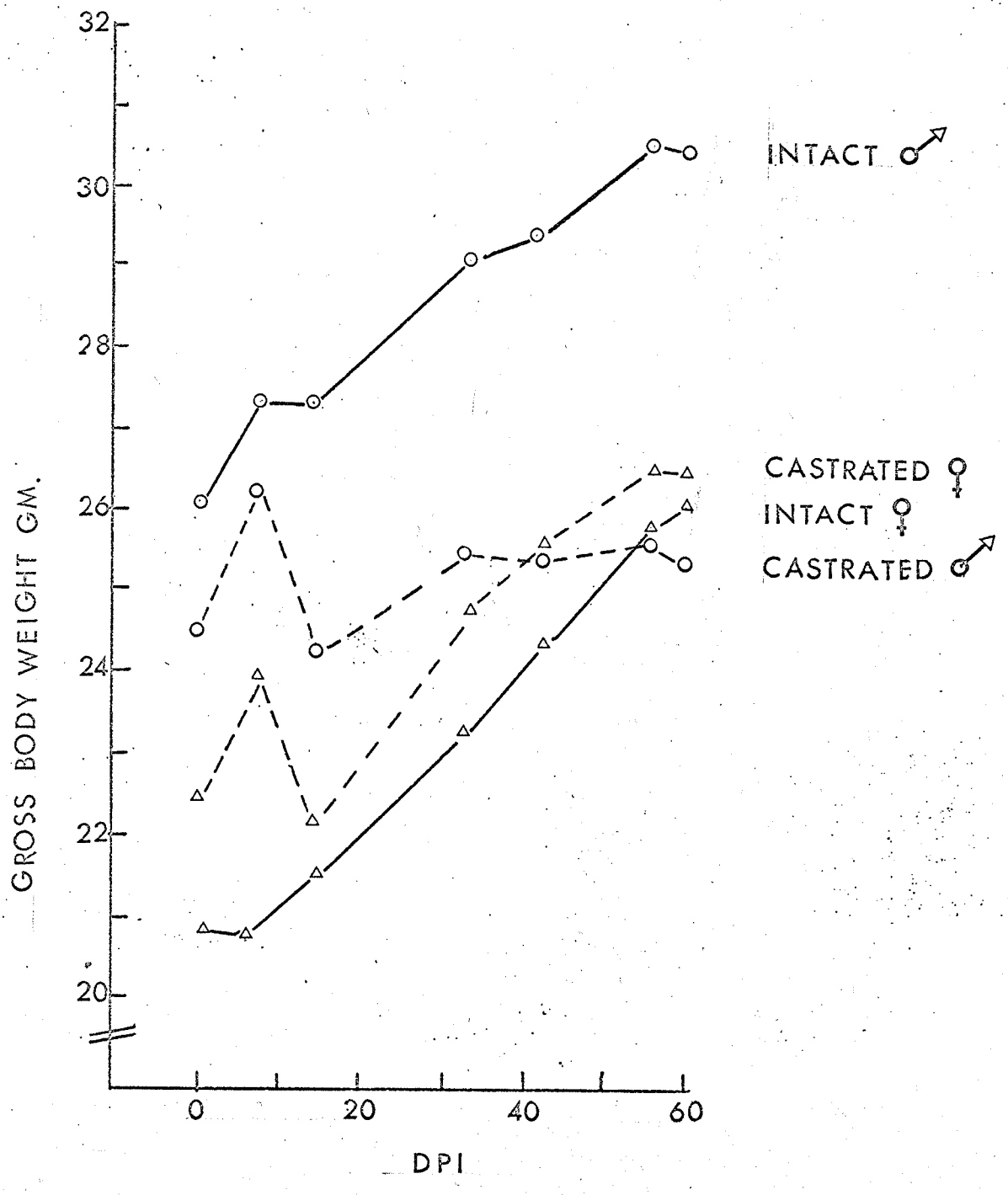
Group I - 20 intact, infected male controls.

Group II - 30 castrated, infected males.

Group III - 20 intact, infected female controls.

Group IV - 30 castrated, infected females.

Castration was performed 7 days prior to infection. The concentration of the cyst material in the inoculum was 31 per cent. The mice were autopsied 69 DPI.


32
30
28
26
24
22
20
GROSS BODY WEIGHT GM.
0
20
40
60
DPI
INTACT ♂
CASTRATED ♀
INTACT ♀
CASTRATED ♂

Cyst weights: In castrated mice the cyst weight was smaller than in the controls (Fig. 6). The difference was highly significant in the males ($P < .001$) and just significant in the females ($P < .05$) (Table VII).

Host reactions are summarized in Table VIII. Mortality in each group was: Group I - 2, Group II - 0, Group III - 1, and Group IV - 1. Splenomegaly was quite significant in all groups. The enlargement of the spleen was greater in the intact females, Group III, than in the intact males, Group I, and in both castrated groups (Group II and IV) splenomegaly was less pronounced than in the controls.

The mean gross body weight of mice in Group I increased and average of 6 gm. during the experiment (Fig. 8) while the net body weight increased only 2.63 gm. Castrated male mice showed an initial increase in mean gross weight in the course of the first 10 days, but after this it remained fairly constant. The net body weight of this group increased only by 0.58 gm. In intact females there was an increase in mean gross weight over 9 gm. while the net body weight gain was only 2.74 gm. Group IV, castrated females, exhibited a gain of close to 7 gm. in mean gross weight with only 2.76 gm. of this being net body weight gain. It is evident that all mice gained in gross weight but only a small amount of this gain can be attributed to net body weight gains. The remaining weight increase was cyst growth.

Experiment 4. This experiment was basically similar to the others except that castration was performed 30 days prior to infection. One hundred, 2-month-old mice were divided into 4 groups.

Group I - 20 intact, infected male controls.

Group II - 30 castrated, infected males.

Group III - 20 intact, infected female controls.

Group IV - 30 castrated, infected females.

Figure 8. Gross body weight changes in castrated mice. Experiment 3.

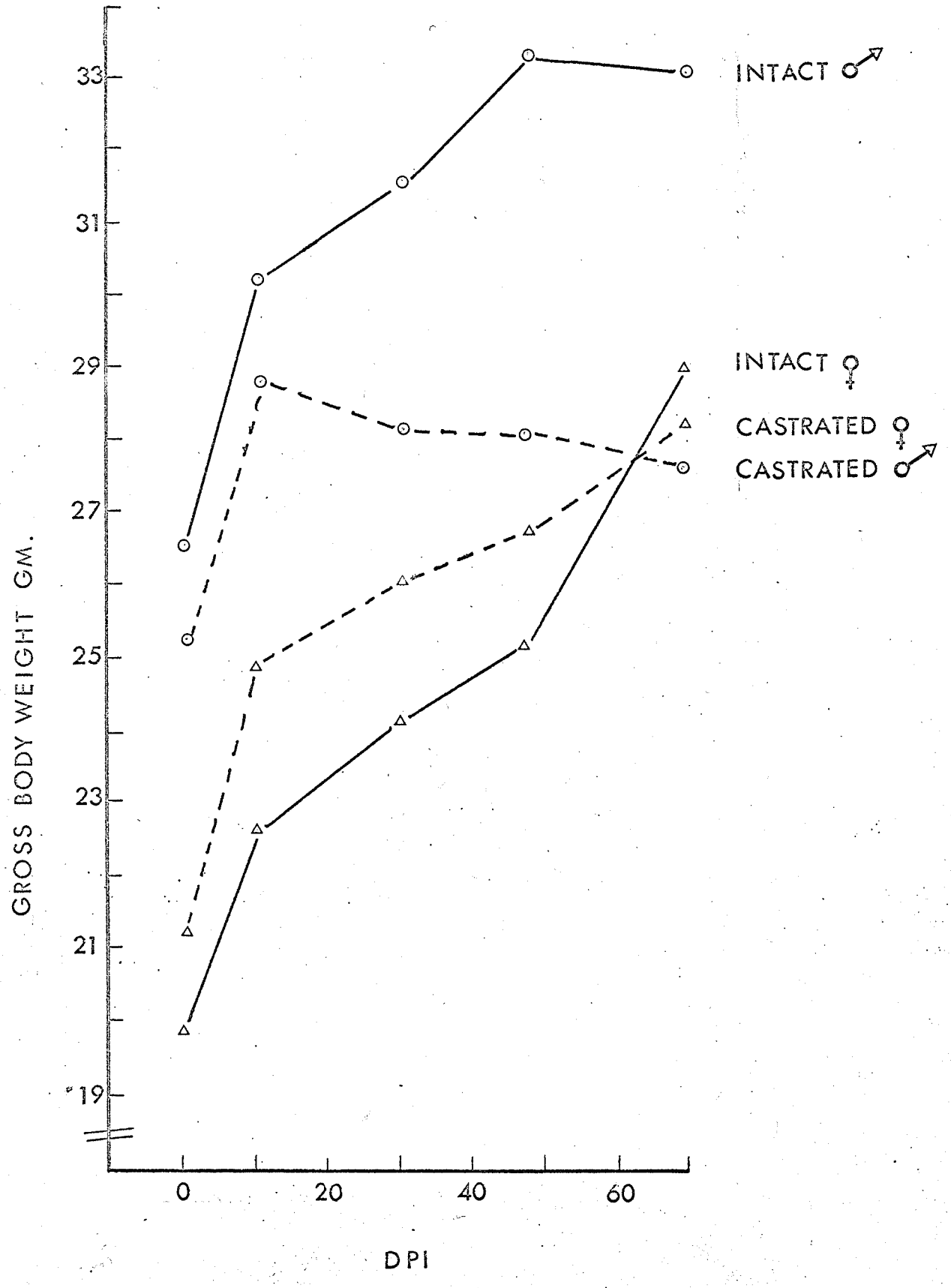

INTACT ♂
INTACT ♀
CASTRATED ♀
CASTRATED ♂
GROSS BODY WEIGHT GM.
33
31
29
27
25
23
21
19
0
20
40
60
DPI

TABLE VIII

Host Responses of Castrated $LD_1F_1$ Mice

| Exp. | Group | Sex | Body Weight at Infection gm. | Net Weight[a] at Autopsy gm. | Weight[b] Change gm. | Spleen Weight gm. |
|---|---|---|---|---|---|---|
| 2 | I control | ♂ | 26.16 | 26.91 | +0.75 | $0.40 \pm 0.04$ |
| | II castrated | ♂ | 24.52 | 24.38 | -0.14 | $0.26 \pm 0.03$ |
| | III control | ♀ | 20.97 | 21.72 | +0.75 | $0.45 \pm 0.04$ |
| | IV castrated | ♀ | 22.54 | 23.64 | +1.10 | $0.44 \pm 0.04$ |
| 3 | I control | ♂ | 26.54 | 29.17 | +2.63 | $0.48 \pm 0.04$ |
| | II castrated | ♂ | 25.26 | 25.84 | +0.58 | $0.28 \pm 0.02$ |
| | III control | ♀ | 19.87 | 22.61 | +2.74 | $0.72 \pm 0.04$ |
| | IV castrated | ♀ | 21.26 | 24.02 | +2.76 | $0.51 \pm 0.04$ |
| 4 | I control | ♂ | 24.81 | 25.28 | +0.47 | $0.48 \pm 0.04$ |
| | II castrated | ♂ | 22.02 | 23.37 | +1.35 | $0.44 \pm 0.04$ |
| | III control | ♀ | 19.13 | 20.02 | +0.89 | $0.68 \pm 0.03$ |
| | IV castrated | ♀ | 21.28 | 21.83 | +0.55 | $0.57 \pm 0.03$ |

[a] Net weight = Gross body weight - Cyst weight

[b] Weight change = Net weight at autopsy - Body weight at infection

Mice were infected with cysts, the concentration in the inoculum being 40 per cent. The mice were autopsied 53 DPI.

Cyst weights in the control groups were the highest observed in all 4 experiments though the experiment was of shorter duration than the others, maybe because concentration of the infectious material was highest of all (Table VII). The difference in mean cyst weights between intact and castrated males was significant ($P < .05$) while that between intact and castrated females was highly significant ($P < .001$).

Host reactions are summarized in Table VIII. Three deaths before discontinuation of the experiment were recorded; one each in Groups I, II and IV. Significant splenomegaly was observed in all groups with Group III showing the greatest spleen enlargement. The splenomegaly was not as great in the castrated groups as in the controls (Table VIII).

The same pattern as in previous experiments was observed when gross body weights were plotted against time (Fig. 9). Group I mice gained over 5 gm., those of Group III over 8 gm. The net body weight increases amounted to only 0.47 gm. and 0.89 gm. respectively. The mean gross body weight of castrated male and female mice increased by an average of about 4 gm. In both groups only about one gram of this was attributable to net body weight gains.

In this experiment some gross weight gains were observed after castration but before infection (Fig. 10). An idea of the effects of castration on body weight were observed along with the normal weight gains made by intact control $LD_1F_1$ mice. Group I, intact males, gained an average of 6 gm. in the 30 days prior to infection, those of Group III and average of 3 gm. Group II, castrated males, gained 4 gm. in the same period. The castrated females, Group IV, gained over 5.5 gm. compared

Figure 9. Gross body weight changes in castrated mice. Experiment 4.

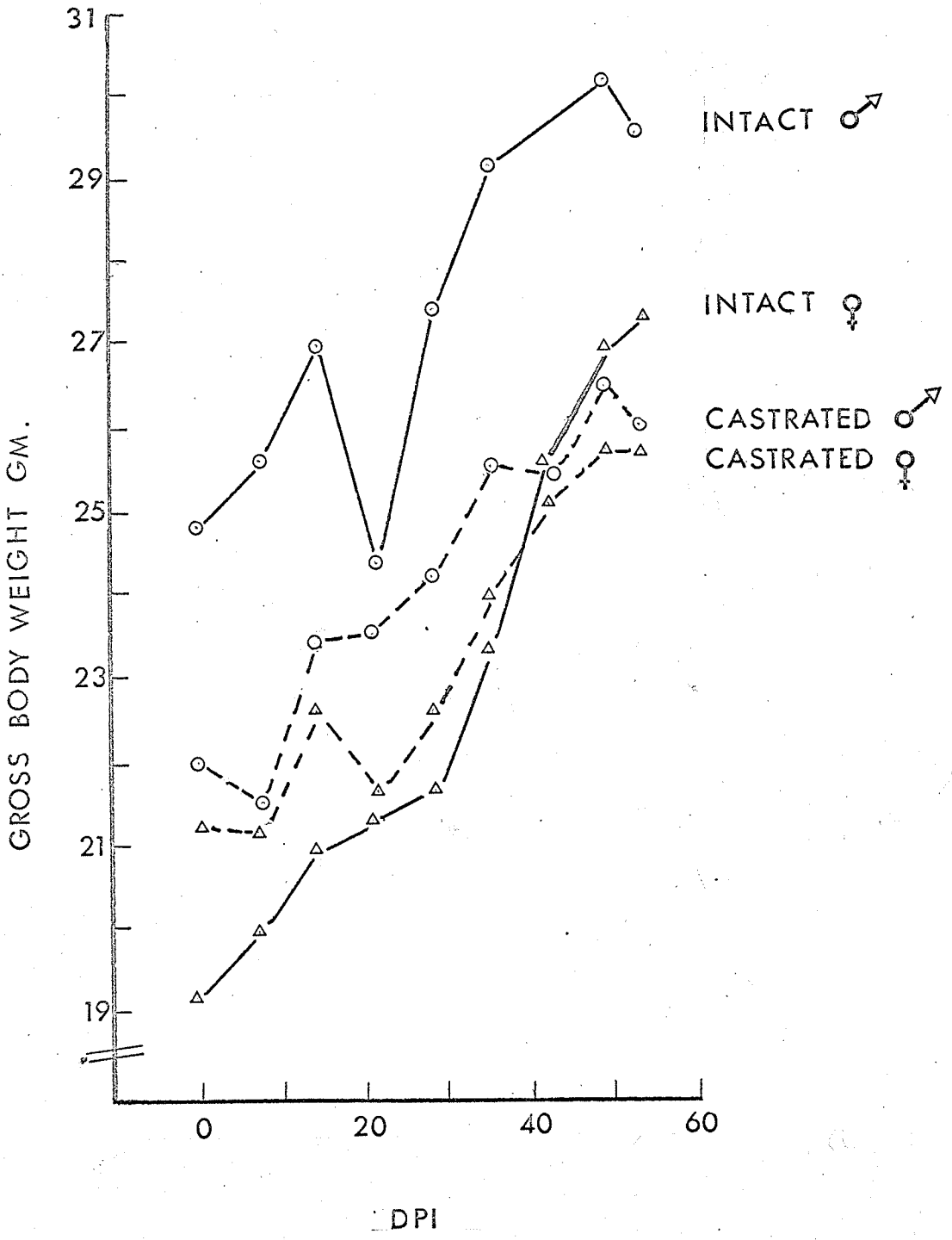
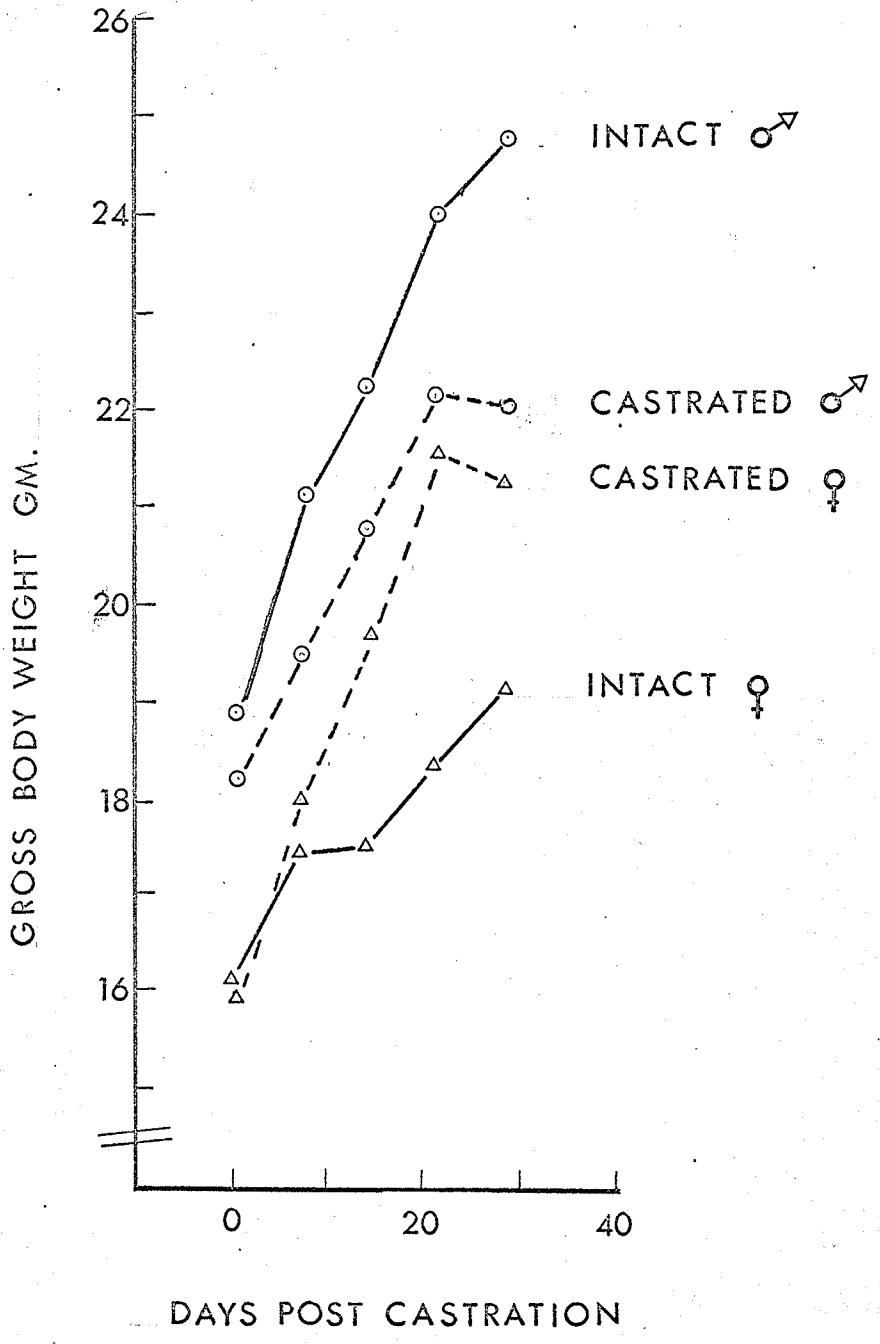

31
29
27
25
23
21
19
GROSS BODY WEIGHT GM.
0
20
40
60
DPI
INTACT ♂
INTACT ♀
CASTRATED ♂
CASTRATED ♀

Figure 10. Gross body weight changes in castrated mice prior to infection. Experiment 4.


26
24
22
20
18
16
GROSS BODY WEIGHT GM.
INTACT ♂
CASTRATED ♂
CASTRATED ♀
INTACT ♀
0
20
40
DAYS POST CASTRATION

to 3 gm. in the controls.

3. Comparison of Cyst Growth Curves in Castrated and Intact $LD_1F_1$ Females.

The possibility that a certain phase of the growth curve of the cysts within the host was altered by castration was examined in the following experiment.

Experiment 5. A total of 60 female $LD_1F_1$ mice were used. Thirty of these were castrated 7 days prior to infection. The others were left as infected, intact controls. All animals were 2 months old when infected with a 26 per cent suspension of cyst material obtained from transfer 60. Autopsy was performed 35, 49 and 60 DPI. Ten controls and 10 castrated animals were autopsied at each interval.

Cyst weights: The castrated group at each of the 3 intervals harboured significantly lesser amounts of cysts than the controls, this difference increasing at each subsequent interval (Table IX). The growth curves of the parasite in terms of cyst weight in both groups were basically sigmoid (Fig. 11). The first phase, that of slow growth, lasted about 35 days in the controls and about 50 days in the castrated animals. The second phase, that of acceleration, occurred between 35 to 50 DPI in the controls and 50 to 60 DPI in the operated group. The phase of fast exponential growth started between 50 to 60 DPI in the controls and lasted to discontinuation of the experiment 60 DPI. This phase had not commenced at this time in the experimental group.

Host reactions: Only one infected control died before the end of the experiment. Enlargement of the spleen over the normal weight of 0.07 gm. was observed in both groups. This enlargement was greater in the controls than in the castrated animals and it was greater in the infections lasting the longest (Table X).

TABLE IX

Growth of Larval E. multilocularis[a] in Castrated Female $LD_1F_1$ Mice

| Group[b] | Castration (days before infection | Number Autopsied | Autopsy DPI[c] | Cyst Weight | | |
|---|---|---|---|---|---|---|
| | | | | gm. | t | P |
| I control | - | 10 | 35 | $1.09 \pm 0.15$ | | |
| II castrated | 7 | 10 | 35 | $0.59 \pm 0.14$ | 2.30 | $< .05$ |
| I control | - | 9 | 49 | $3.29 \pm 0.81$ | | |
| II castrated | 7 | 10 | 49 | $1.08 \pm 0.32$ | 2.61 | $< .02$ |
| I control | - | 10 | 60 | $8.53 \pm 1.10$ | | |
| II castrated | 7 | 10 | 60 | $3.28 \pm 0.75$ | 3.84 | $< .01$ |

[a]Concentration of infectious material = 26%

[b]Mice were 2 months old at infection

[c]Days post infection

TABLE X

Host Responses of Castrated Female $LD_1F_1$ Mice

| Group | Autopsy DPI[a] | Body Weight at Infection gm. | Net Weight[b] at Autopsy gm. | Weight[c] Change gm. | Spleen Weight gm. |
|---|---|---|---|---|---|
| I control | 35 | 17.00 | 22.55 | +5.55 | $0.30 \pm 0.02$ |
| II castrated | 35 | 18.47 | 23.65 | +5.18 | $0.23 \pm 0.02$ |
| I control | 49 | 17.00 | 20.22 | +3.22 | $0.35 \pm 0.07$ |
| II castrated | 49 | 18.47 | 23.94 | +3.94 | $0.22 \pm 0.04$ |
| I control | 60 | 17.00 | 20.92 | +3.92 | $0.69 \pm 0.06$ |
| II castrated | 60 | 18.47 | 22.52 | +4.05 | $0.49 \pm 0.07$ |

[a] Days post infection

[b] Net weight = Gross body weight - Cyst weight

[c] Weight change = Net weight at autopsy - Body weight at infection

Figure 11. Growth curve of E. multilocularis cysts in castrated female mice.

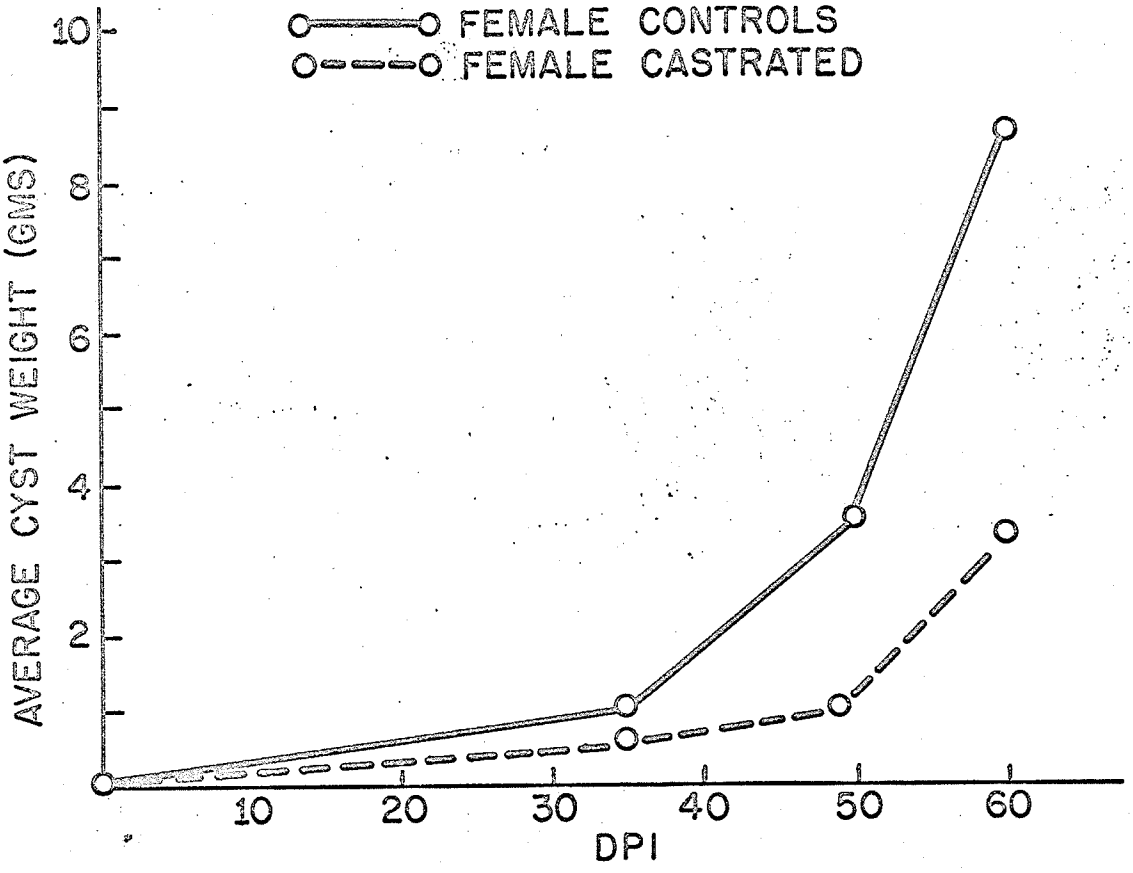
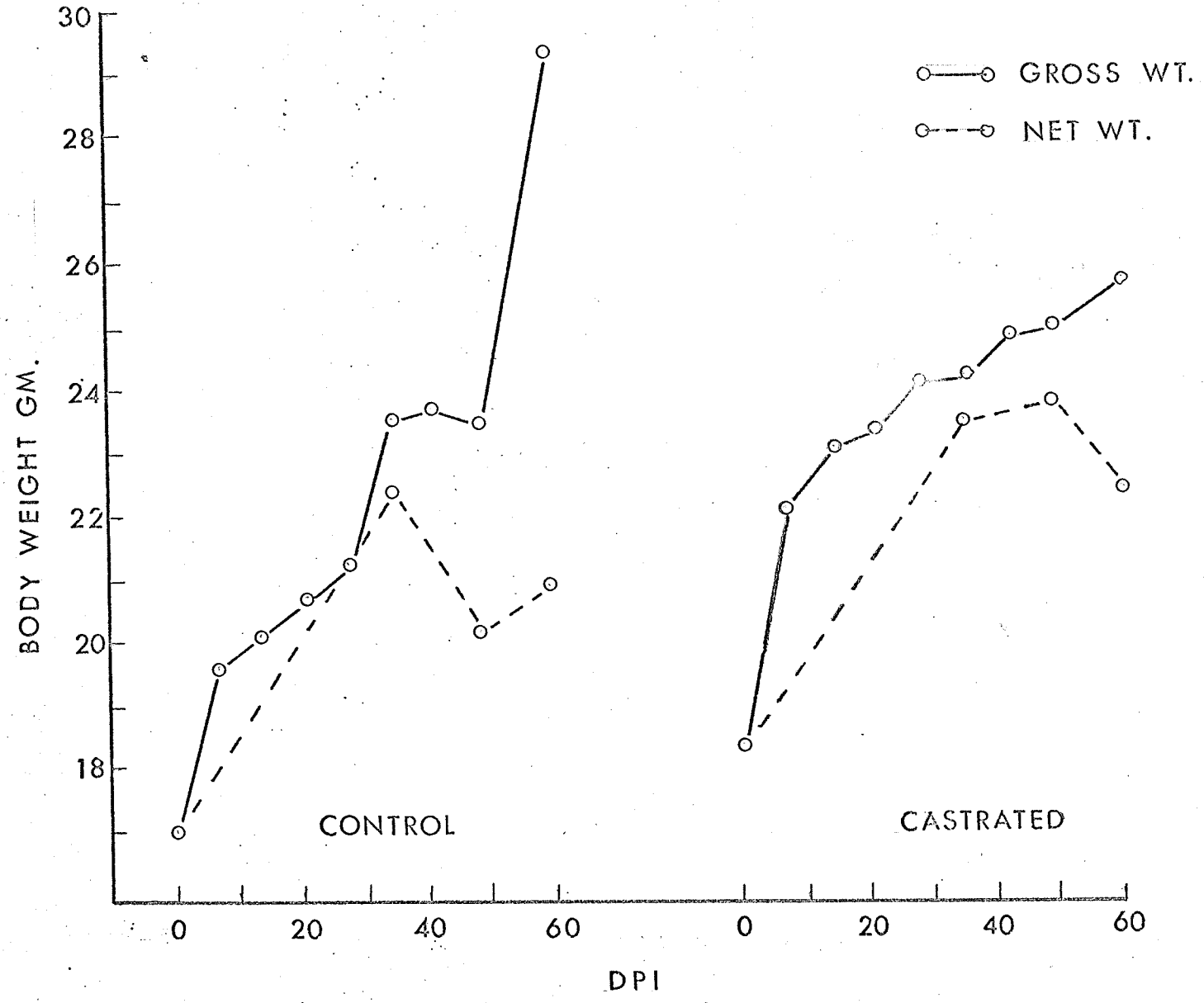

FEMALE CONTROLS
FEMALE CASTRATED
AVERAGE CYST WEIGHT (GMS)
10
8
6
4
2
10
20
30
40
50
60
DPI

Figure 12. Comparison of gross and net body weight changes in castrated female mice.


30
28
26
24
22
20
18
BODY WEIGHT GM.
GROSS WT.
NET WT.
CONTROL
CASTRATED
0
20
40
60
0
20
40
60
DPI

Comparisons between the gross and net body weight changes were made at each autopsy. The gross body weight of the controls increased steadily throughout the experiment until the autopsy at 60 DPI (Fig. 12). This increase amounted to an average of over 13 gm./mouse. The net body weight increased until 35 DPI and then began to decline slightly as cyst growth began to accelerate.

In the castrated females the gross body weight increase over the 60 day period of infection averaged about 8 gm. The net body weight increased until about 35 DPI, tapered off slightly 49 DPI and then began dropping.

Hormone Treatment

Cyst growth is significantly greater in the female than in the male host and castration depresses this growth in both sexes. These two observations made in earlier experiments indicate that sex hormones may be influencing the growth of cysts. To test this hypothesis and androgen, an estrogen and a gonadotrophin were injected into intact and castrated $LD_1F_1$ mice of both sexes which were infected intraperitoneally with cysts. The hormones given were:

1. Testosterone propionate (Orchisterone P, Charles E. Frosst & Co.).
2. Estradiol benzoate (Oestroform, British Drug Houses).
3. Chorionic gonadotrophin (Antuitrin-S, Parke Davis & Co.).

Three series of experiments were made. These were:

1. Experiment 1 and 2. Treatment of intact and castrated mice with testosterone propionate and with estradiol benzoate.

2. Experiment 3 and 4. Treatment of intact and castrated mice with testosterone propionate, with estradiol benzoate and with chorionic gonadotrophin.

3. Experiment 5. Treatment of castrated female mice with testosterone propionate and with estradiol benzoate.

In all cases $LD_1F_1$ hybrid mice were used and castration took place 7 days prior to infection.

1. Experiment 1 and 2. The effects of testosterone propionate and of estradiol benzoate on cyst growth in intact and castrated male and female mice were studied.

Experiment 1. A total of 82 female $LD_1F_1$ mice, 4-month-old, were divided into 5 groups.

Group I - 14 intact, infected controls.

Group II - 17 intact, infected, injected with testosterone.

Group III - 17 intact, infected, injected with estradiol.

Group IV - 16 castrated, infected controls.

Group V - 18 castrated, infected and injected with estradiol.

The 62nd cotton rat transfer was the source of the infectious material and the cyst concentration in the inoculum was 20 per cent. Hormone treatment began the day following infection and injections were given twice a week for 5 weeks. The doses of hormones were: Testosterone propionate, 8.0 $\mu$g/g and estradiol benzoate, 4.0 $\mu$g/g. Autopsy was performed 54 DPI. Results are summarized in Table XI and XII.

Cyst weights: Both the testosterone and the estradiol-treated groups had heavier cyst loads than the intact controls (Fig. 13). Control mice had a mean cyst weight of $2.66 \pm 0.41$ gm., the testosterone-treated a mean cyst weight of $3.90 \pm 0.54$ gm. This difference was statistically significant ($P < .05$). The cyst weight in estradiol-treated mice was

Figure 13. Growth of cysts in hormone-treated mice. Experiment 1 and 2. Numbers over columns indicate the number of mice autopsied.

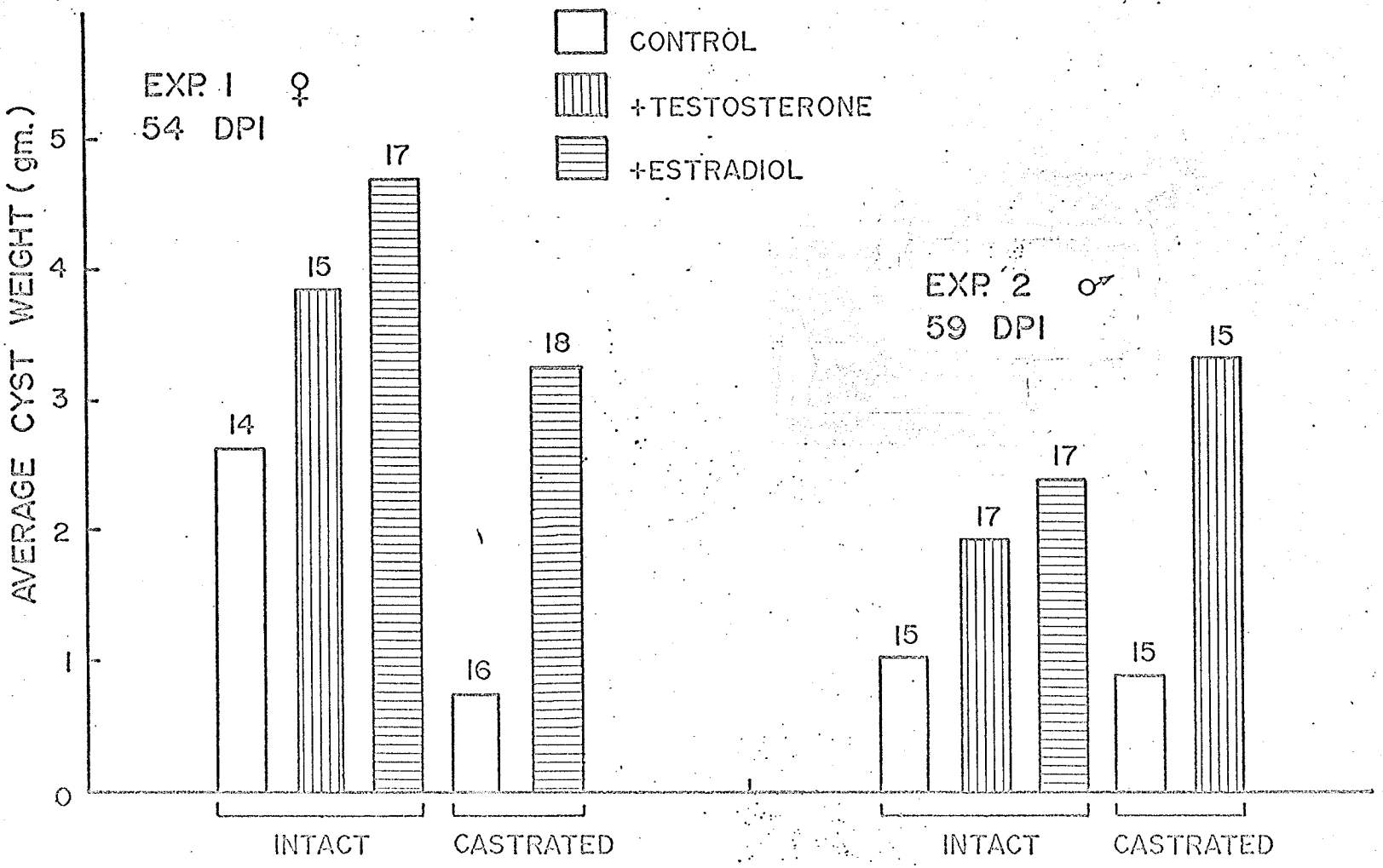

CONTROL
+TESTOSTERONE
+ESTRADIOL
EXP. 1 ♀
54 DPI
AVERAGE CYST WEIGHT (gm.)
0
1
2
3
4
5
14
15
17
16
18
INTACT
CASTRATED
EXP. 2 ♂
59 DPI
15
17
17
15
15
INTACT
CASTRATED

TABLE XI

Growth of Larval Echinococcus multilocularis in Hormone-treated $LD_1F_1$ Mice

| Exp. | Group | Sex | Castration (days before infection) | Cyst Conc. % | Number Autopsied | Autopsy DPI[a] | Cyst Weight gm. |
|---|---|---|---|---|---|---|---|
| 1 | I intact control | ♀ | - | 20 | 14 | 54 | $2.66^{\pm}$ 0.41 |
| | II intact + testosterone | ♀ | - | 20 | 15 | 54 | $3.90^{\pm}$ 0.54 |
| 4 month old $LD_1F_1$ | III intact + estradiol | ♀ | - | 20 | 17 | 54 | $4.70^{\pm}$ 0.51 |
| | IV castrated control | ♀ | 7 | 20 | 16 | 54 | $0.72^{\pm}$ 0.16 |
| | V castrated + estradiol | ♀ | 7 | 20 | 18 | 54 | $3.26^{\pm}$ 0.62 |
| 2 | I intact control | ♂ | - | 40 | 15 | 59 | $1.04^{\pm}$ 0.23 |
| | II intact + testosterone | ♂ | - | 40 | 17 | 59 | $1.96^{\pm}$ 0.39 |
| 4 month old $LD_1F_1$ | III intact + estradiol | ♂ | - | 40 | 17 | 59 | $2.35^{\pm}$ 0.45 |
| | IV castrated control | ♂ | 7 | 40 | 15 | 59 | $0.85^{\pm}$ 0.33 |
| | V castrated + testosterone | ♂ | 7 | 40 | 15 | 59 | $3.42^{\pm}$ 0.83 |
| 3 | I intact control | ♀ | - | 40 | 15 | 44 | $2.43^{\pm}$ 0.33 |
| | II intact + testosterone | ♀ | - | 40 | 14 | 44 | $3.11^{\pm}$ 0.37 |
| 3 month old $LD_1F_1$ | III intact + estradiol | ♀ | - | 40 | 14 | 44 | $3.74^{\pm}$ 0.69 |
| | IV intact + HCG | ♀ | - | 40 | 16 | 44 | $2.11^{\pm}$ 0.37 |
| | V castrated control | ♀ | 7 | 40 | 15 | 44 | $1.16^{\pm}$ 0.28 |
| | VI castrated + estradiol | ♀ | 7 | 40 | 15 | 44 | $2.64^{\pm}$ 0.37 |
| 4 | I intact control | ♂ | - | 30 | 15 | 47 | $1.81^{\pm}$ 0.30 |
| | II intact + testosterone | ♂ | - | 30 | 14 | 47 | $2.50^{\pm}$ 0.46 |
| 3 month old $LD_1F_1$ | III intact + estradiol | ♂ | - | 30 | 14 | 47 | $2.05^{\pm}$ 0.25 |
| | IV intact + HCG | ♂ | - | 30 | 15 | 47 | $2.02^{\pm}$ 0.52 |
| | V castrated control | ♂ | 7 | 30 | 15 | 47 | $1.74^{\pm}$ 0.46 |
| | VI castrated + testosterone | ♂ | 7 | 30 | 14 | 47 | $5.82^{\pm}$ 0.50 |

[a] Days post infection

TABLE XII

Statistical Comparisons of Cyst Growth in Hormone-treated Mice

| Exp. | Group | t | P |
|---|---|---|---|
| 1 | I intact control | | |
| | II intact + testosterone | 1.76 | $<.05$ |
| | III intact + estradiol | 3.06 | $<.01$ |
| | IV castrated control | | |
| | V castrated + estradiol | 3.61 | $<.01$ |
| | I intact control | 4.48 | $<.001$ |
| 2 | I intact control | | |
| | II intact + testosterone | 1.99 | $<.05$ |
| | III intact + estradiol | 2.49 | $<.02$ |
| | IV castrated control | | |
| | V castrated + testosterone | 2.88 | $<.01$ |
| | I intact control | 0.47 | $>.05$ |
| 3 | I intact control | | |
| | II intact + testosterone | 1.40 | $>.05$ |
| | III intact + estradiol | 1.74 | $>.05$ |
| | IV intact + HCG | 0.64 | $>.05$ |
| | V castrated control | | |
| | VI castrated + estradiol | 3.15 | $<.01$ |
| | I intact control | 2.91 | $<.01$ |
| 4 | I intact control | | |
| | II intact + testosterone | 1.25 | $>.05$ |
| | III intact + estradiol | 0.61 | $>.05$ |
| | IV intact + HCG | 0.34 | $>.05$ |
| | V castrated control | | |
| | VI castrated + testosterone | 5.96 | $<.001$ |
| | I intact control | 0.12 | $>.05$ |

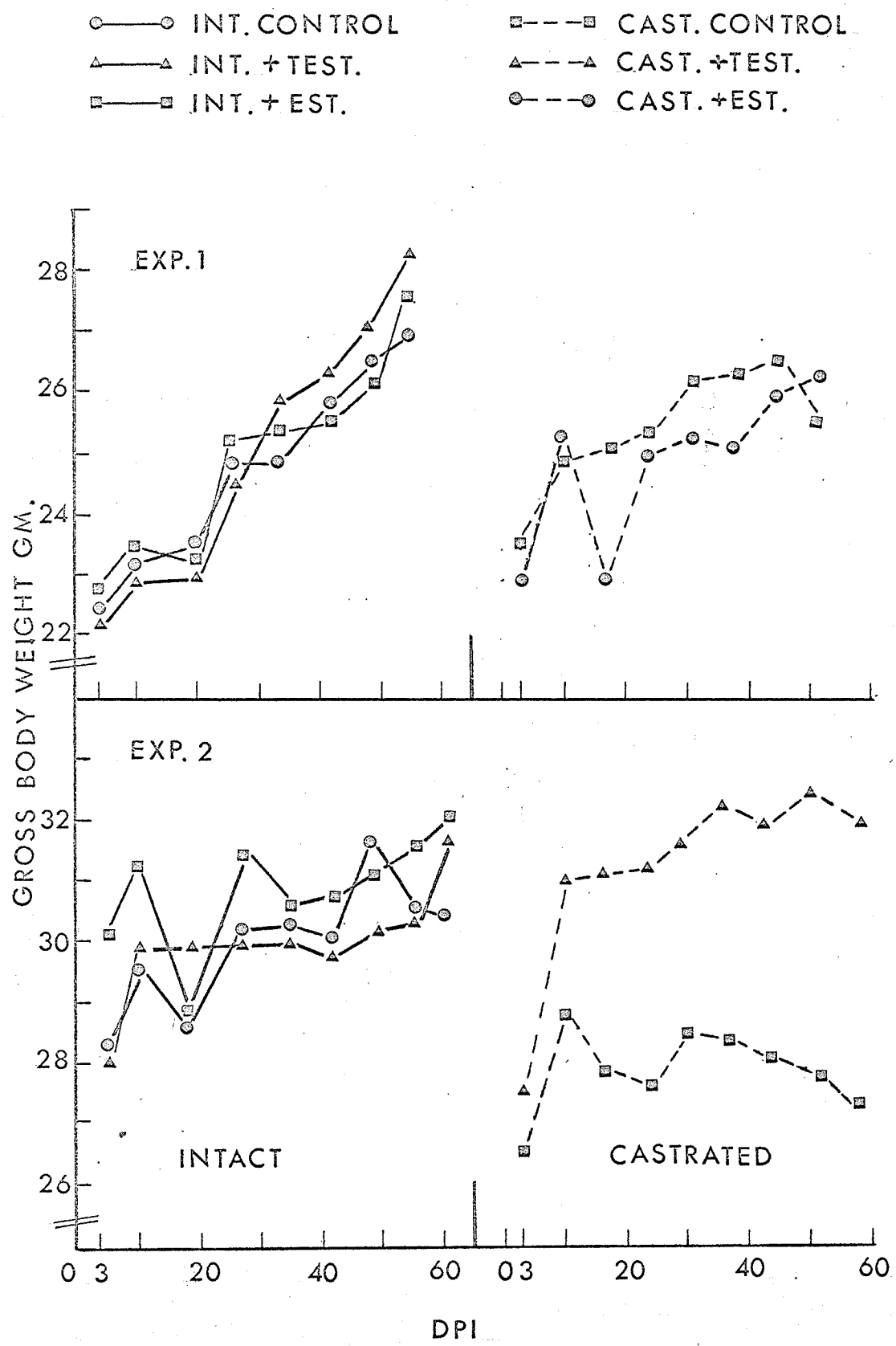
$4.70 \pm 0.51$ gm., thus also significantly more than in the controls ($P < .01$). The castrated controls, Group IV, had significantly lesser amounts of cysts than the intact controls, Group I ($P < .001$). Estradiol benzoate significantly affected the growth of cysts in castrated mice, the mean cyst weight of mice in this group being significanlty higher than in the castrated controls ($P \leq .01$).

Protoscoleces were found in all groups. This was unexpected since the infection was only 54 days old and protoscoleces do not usually appear in mice until after 3 months. The protoscoleces difinitely developed in the mouse and were not transferred from the cotton rat donor, since very immature forms were present.

Host reactions in experimental mice are summarized in Table XIII. Deaths before the termination fo the experiment were minimal with 2 mice lost from Group II. None of the mice appeared to be emaciated at the time of autopsy, 54 DPI. Mice of all 5 groups gained in gross body weight, (Fig. 14). The changes in body weight were quite similar in mice of the 3 intact groups (Groups I, II and III). The castrated controls and the treated, castrated animals also gained in gross body weight but to a lesser extent than the intact mice. Both the intact and testosterone-treated mice showed an increase in net body weight, but this was not the case in estradiol-treated mice (Table XIII). The castrated controls gained an average of 0.92 gm. while castrated mice treated with estradiol decreased in weight by 0.18 gm. in the course of the experiment.

Spleen enlargment was observed in all 5 groups with a maximum of up to 6 times the normal spleen weight of 0.07 gm. The kidney in a female mouse weighs about 0.24 gm. Both testosterone and estradiol-treated mice had heavier kidneys than the controls. Castrated mice had a significantly

Figure 14. Gross body weight changes in hormone-treated mice. Experiment 1 and 2.


INT. CONTROL
INT. + TEST.
INT. + EST.
CAST. CONTROL
CAST. + TEST.
CAST. + EST.
EXP. 1
EXP. 2
GROSS BODY WEIGHT GM.
28
26
24
22
32
30
28
26
INTACT
CASTRATED
0 3 20 40 60
0 3 20 40 60
DPI

lighter kidney, 0.20 gm. This weight increased to normal in estradiol-treated castrated mice. The weight of the ovaries was not affected by hormone administration, but that of the uterus was markedly influenced. Castration led to a large decrease in the weight of the uterus, the differences in weight between the castrated controls and the intact controls being significant ($P<.001$). Testosterone propionate did not influence the weight of the uterus but in estradiol benzoate-treated mice this weight increased in relation to that in the controls ($P<.001$). Castrated mice treated with estradiol had heavier uteri than both the castrated and the intact controls.

Experiment 2 was similar to Experiment 1, but male $LD_1F_1$ mice were used instead of females. They were 4 months of age at infection. A total of 79 mice were divided into 5 groups.

Group I - 15 intact, infected controls.

Group II - 17 intact, infected, injected with testosterone.

Group III - 17 intact, infected, injected with estradiol.

Group IV - 15 castrated, infected controls.

Group V - 15 castrated, infected and injected with testosterone.

Mice were infected with cyst material, diluted to a concentration of 40 per cent. Hormone treatment began the day following infection and injections were given twice a week for 5 weeks. The doses were: testosterone propionate, 8.0$\mu$g/g, estradiol benzoate, 4.0$\mu$g/g. Autopsy was performed 59 DPI. Results are summarized in Table XI and XII.

Cyst weights: Testosterone-treated mice had significantly larger cysts than the intact controls ($P<.05$). This was also the case in estradiol-treated mice (Fig. 13), which had larger cysts than both the

TABLE XIII

Host Responses of Hormone-treated $LD_1F_1$ Mice

| Exp. | Group | Sex | Weight at Infection gm. | Net[a] Weight at Autopsy gm. | Weight[b] Change gm. | Spleen Weight gm. | Kidney[c] Weight gm. | Ovaries[d] Weight gm. | Uterus[e] Weight gm. | Testes[f] Weight gm. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I intact control | | 22.66 | 24.66 | +1.50 | 0.47 | 0.24 | 0.06± 0.003 | 0.16± 0.010 | |
| | II intact + testosterone | | 22.38 | 24.25 | +1.87 | 0.53 | 0.30 | 0.05± 0.003 | 0.17± 0.009 | |
| | III intact + estradiol | | 22.76 | 22.89 | +0.13 | 0.54 | 0.26 | 0.06± 0.003 | 0.24± 0.010 | |
| | IV castrated control | | 23.63 | 24.55 | +0.92 | 0.23 | 0.20 | | 0.09± 0.004 | |
| | V castrated + estradiol | | 22.94 | 22.66 | -0.18 | 0.42 | 0.25 | | 0.21± 0.010 | |
| 2 | I intact control | | 28.47 | 29.67 | +1.20 | 0.26 | 0.33 | | | 0.19± 0.006 |
| | II intact + testosterone | | 28.29 | 29.73 | +1.44 | 0.34 | 0.36 | | | 0.17± 0.004 |
| | III intact + estradiol | | 30.14 | 29.27 | -0.87 | 0.42 | 0.32 | | | 0.15± 0.006 |
| | IV castrated control | | 26.33 | 26.36 | +0.03 | 0.22 | 0.23 | | | |
| | V castrated + testosterone | | 27.56 | 28.25 | +0.69 | 0.44 | 0.32 | | | |
| 3 | I intact control | | 18.50 | 21.71 | +3.21 | 0.35 | 0.24 | 0.06± 0.002 | 0.18± 0.015 | |
| | II intact + testosterone | | 19.73 | 22.04 | +2.31 | 0.33 | 0.28 | 0.04± 0.004 | 0.14± 0.005 | |
| | III intact + estradiol | | 19.80 | 22.60 | +2.80 | 0.34 | 0.26 | 0.07± 0.010 | 0.23± 0.010 | |
| | IV intact + HCG | | 20.06 | 23.19 | +3.13 | 0.27 | 0.24 | 0.06± 0.004 | 0.15± 0.013 | |
| | V castrated control | | 20.26 | 24.12 | +3.86 | 0.25 | 0.21 | | 0.08± 0.008 | |
| | VI castrated + estradiol | | 20.57 | 23.36 | +2.79 | 0.48 | 0.27 | | 0.24± 0.007 | |
| 4 | I intact control | | 26.73 | 27.87 | +1.14 | 0.49 | 0.30 | | | 0.20± 0.010 |
| | II intact + testosterone | | 24.80 | 27.18 | +2.38 | 0.43 | 0.37 | | | 0.18± 0.007 |
| | III intact + estradiol | | 26.06 | 26.59 | +0.53 | 0.44 | 0.30 | | | 0.20± 0.005 |
| | IV intact + HCG | | 25.27 | 26.69 | +1.42 | 0.40 | 0.31 | | | 0.19± 0.008 |
| | V castrated control | | 22.53 | 23.09 | +0.56 | 0.30 | 0.20 | | | |
| | VI castrated + testosterone | | 24.33 | 24.35 | +0.02 | 0.46 | 0.34 | | | |

[a]Net weight = Gross body wt. - Cyst wt. [b]Weight change = Net weight at autopsy - body wt. at infection

[c]Right kidney [d]Both ovaries [e]Both horns of uterus [f]Both testes

testosterone-treated mice and the controls ($P < .02$). Castration did not significantly affect the cyst growth. However, the testosterone-treated, castrated mice had larger cysts than the controls ($P < .01$). Scoleces were present only in the intact controls (Group I).

Host reactions are summarized in Table XIII. No mortality was observed in the course of the experiment. The gross body weight of the mice in Groups I, II and III increased slightly (Fig. 14). Group IV mice showed an initial increase in gross body weight of over 2 gm. with a subsequent slight decline. Gross body weight of Group V mice (castrated controls) increased steadily throughout the experiment. The net body weight increased in Groups I and II by 1.20 gm. and 1.44 gm. respectively. In Group III it decreased by 0.87 gm. Group IV mice did not change substantially in net weight from the beginning to the end of the experiment while in Group V the weight increased by 0.69 gm.

Enlargment of the spleen was observed in each group with the greatest increases being in mice with larger cysts. Values up to 6 times the normal spleen weight were recorded. The weight of the kidneys was affected both by castration and by hormone treatment. This weight in a normal male was about 0.30 gm. An increase in weight was observed in testosterone-treated mice but not in those treated with estradiol. Castrated males had a significantly lighter kidney than normal and testosterone-treated, castrated males had normal kidney weights. The weight of both testes was depressed below the normal of 0.20 gm. by both testosterone and estradiol treatment.

2. Experiment 3 and 4. This set of experiments was basically a repetition of those of the first series, except that another hormone, human chorionic

gonadotrophin (HCG) was used. The gonadotrophin stimulates the production of sex hormones, and in this way the effects of endogenous sex hormone on cyst growth could be studied.

Experiment 3. Ninety-one female $LD_1F_1$ hybrids, 3 months old at infection, were used in this experiment. They were divided into 6 groups.

Group I - 15 intact, infected controls.

Group II - 15 intact, infected, injected with testosterone.

Group III - 15 intact, infected, injected with estradiol.

Group IV - 16 intact, infected, injected with HCG.

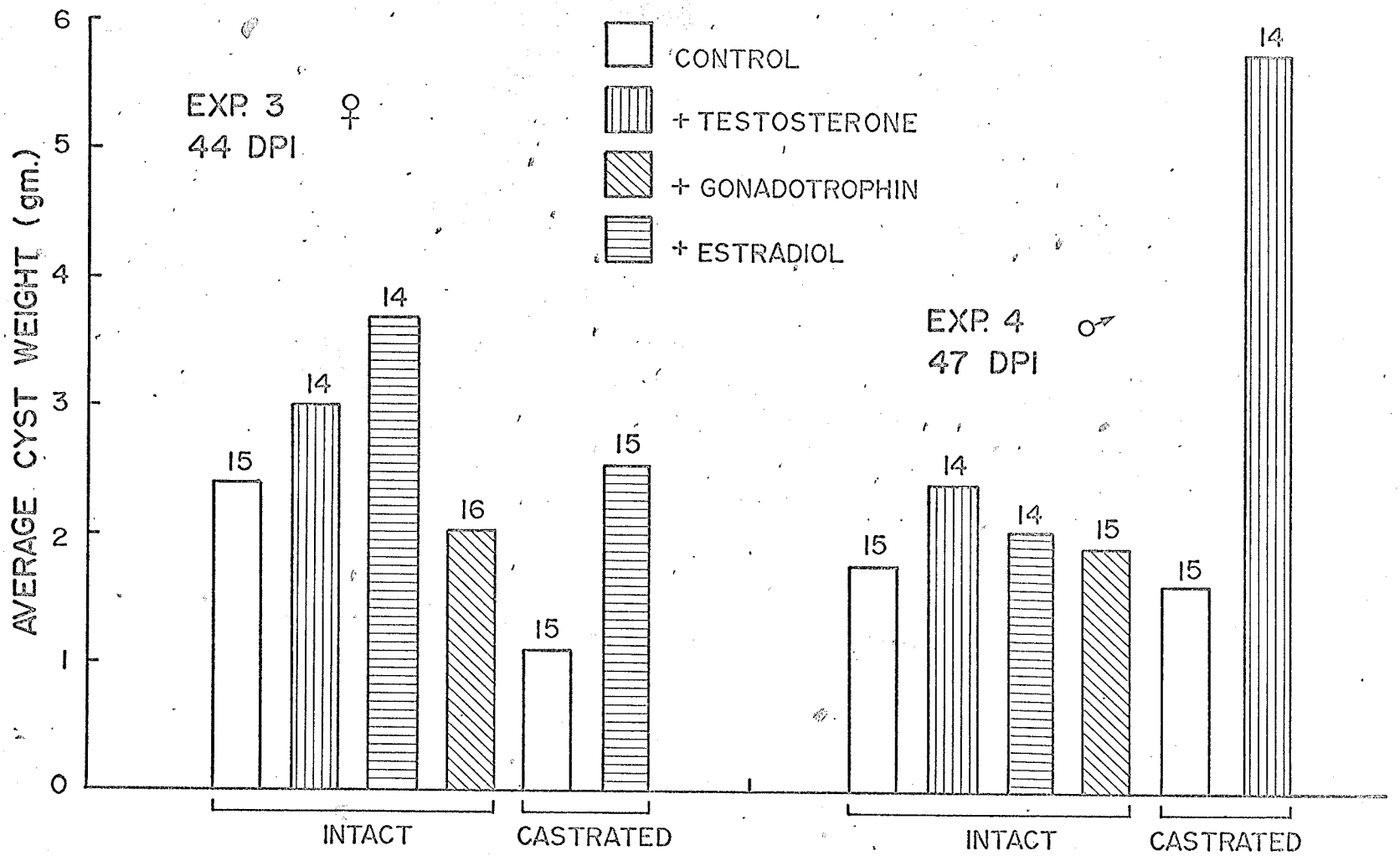
Group V - 15 castrated, infected controls.

Group VI - 15 castrated, infected and injected with estradiol.

The mice were infected with cysts, the concentration of the material in the inoculum being 40 per cent. Hormone injections began the day following infection. The dose of testosterone propionate was 8.0$\mu$g/g, of estradiol benzoate 4.0$\mu$g/g and of chorionic gonadotrophin 1.6 I.U./g. Each was given twice a week for 5 weeks.

Cyst weights are recorded in Table XI. Both testosterone and estradiol-treated groups had larger cysts than the intact controls (Fig. 15), but in neither case was the difference significant (Table XII). Mice treated with gonadotrophin had a smaller cyst load than the controls, but the difference was not significant. The castrated controls had lower cyst weights than the intact controls and the difference was highly significant ($P < .01$). Also, estradiol-treated castrated mice had significantly higher cyst weights than the castrated controls ($P < .01$). The size of cysts in the estradiol-treated, castrated group was equal to that in the intact controls. Protoscoleces were found in cysts from all 6 groups.

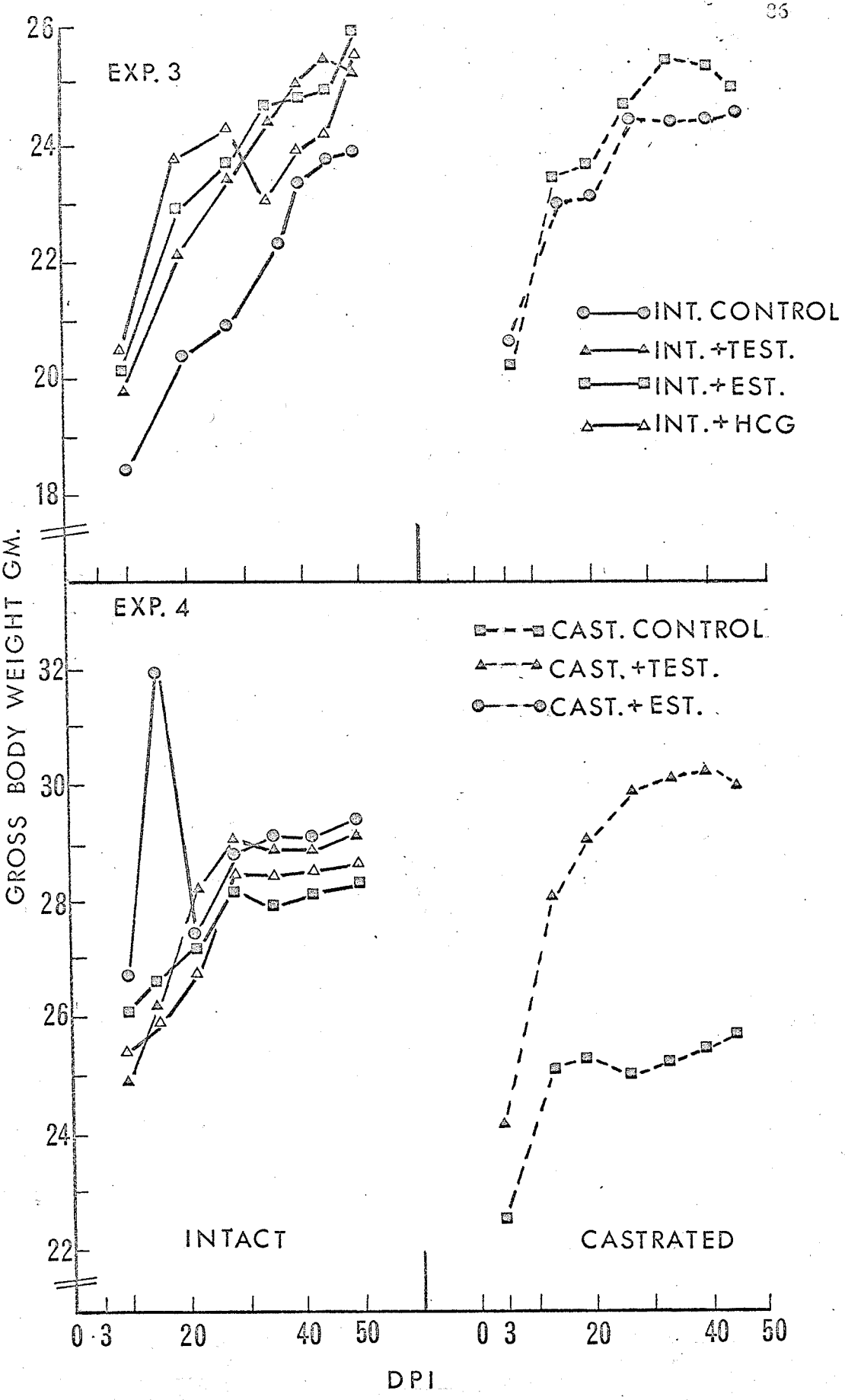
Figure 15. Growth of cysts in hormone-treated mice. Experiment 3 and 4. Numbers over columns indicate numbers of mice autopsied.


AVERAGE CYST WEIGHT (gm.)
0
1
2
3
4
5
6
EXP. 3 ♀
44 DPI
CONTROL
+ TESTOSTERONE
+ GONADOTROPHIN
+ ESTRADIOL
15
14
14
16
15
15
EXP. 4 ♂
47 DPI
15
14
14
15
15
14
INTACT
CASTRATED
INTACT
CASTRATED

Host reactions: Two mice died prior to termination of the experiment, one in Group II and one in Group III. No apparent emaciation was observed in any of the mice at the time of autopsy. The mean gross body weight of all mice increased steadily throughout the experiment. In fact, the growth curves in all groups are quite similar (Fig. 16). In contrast to the previous experiments, substantial net body weight gains were observed in all groups (Table XIII). Spleen enlargement in infected mice was similar to that observed in previous experiments. The weight of the kidneys was affected by castration and hormone treatment. The weight of the kidneys in the intact female controls was 0.24 gm. This weight was greater in Group II ($P < .01$), but not affected in Groups III and IV. Castration depressed the kidney weight to 0.21 gm. and this depression was highly significant ($P < .001$). Estradiol treatment increased the weight of the kidney in castrated animals slightly above the normal value.

The ovaries in testosterone-treated mice weighed significantly less than those of the controls ($P < .001$). They were not affected in estradiol or HCG-treated mice. The uterus was much more affected than the ovaries. The weight of the uterus in the testosterone-treated mice was less than that in the controls ($P < .05$). In the estradiol-treated group the weight was greater than in the controls ($P \leq .05$). Uterine weight was not affected by HCG treatment. Castration significantly reduced the weight of the uterus from that in the intact controls ($P < .001$), and in castrated, estradiol-treated mice the estrogen restored this weight to normal.

Experiment 4. This experiment was similar to the previous one except that male mice were used. A total of 90, 3-month-old, male $LD_1F_1$ mice were


EXP. 3
INT. CONTROL
INT. + TEST.
INT. + EST.
INT. + HCG
EXP. 4
CAST. CONTROL
CAST. + TEST.
CAST. + EST.
GROSS BODY WEIGHT GM.
26
24
22
20
18
32
30
28
26
24
22
INTACT
CASTRATED
0 3
20
40
50
0 3
20
40
50
DPI

divided into 6 groups.

Group I - 15 intact, infected controls.

Group II - 15 intact, infected, injected with testosterone.

Group III - 15 intact, infected, injected with estradiol.

Group IV - 15 intact, infected, injected with HCG.

Group V - 15 castrated, infected controls.

Group VI - 15 castrated, infected and injected with testosterone.

The 63rd cotton rat transfer served as the source of the inoculum and mice were injected intraperitoneally with a 30 per cent suspension of cyst material. Hormone treatment began on the day following infection and 2 injections were given each week for 5 weeks. Testosterone propionate was given in a dose of 8.0 µg/g, estradiol benzoate, 4.0 µg/g and chorionic gonadotrophin, 1.6 I. U./g. Autopsy was performed 47 DPI.

Cyst weights: In the testosterone, estradiol and gonadotrophin-treated groups, the cysts were slightly larger than in the controls (Fig. 15), but in neither were the differences significant (Table XI and XII). There was also no significant difference in cyst size between the intact and castrated controls. Testosterone treatment of castrated males resulted in a large increase in cyst size over that in both the castrated and intact controls as well as all the intact, hormone-treated groups. These differences were significant at the P = .001 level in all cases. Protoscoleces were found in cysts from Groups II, IV, V and VI.

Host reactions are summarized in Table XII. One death in each of Groups II, III and VI occurred. The mean gross body weight of each group increased in a similar manner to those in Experiment 2. Mice of all 4 intact groups increased in weight and the weight curves were very similar in all

of them. Group V gained in weight slightly during the first week, while Group VI increased in weight considerably (Fig. 16). Net weight gains were observed in all groups. These amounted to 1.14 gm. in Group I, 2.38 gm. in Group II, 0.53 gm. in Group III, 1.42 gm. in Group IV, 0.56 gm. in Group V but only to 0.02 gm. in Group VI. Splenomegaly was observed in all groups with values up to 7 times the normal weight of 0.07 gm. The weight of the kidneys was also influenced. The normal weight of the kidneys in male mice was about 0.30 gm. This weight was not affected by estradiol or HCG but was increased in mice treated with testosterone to 0.37 gm. Castration lowered this weight to 0.20 gm. but it was brought back to normal in castrated mice treated with testosterone.

3. Experiment 5. This experiment was made to compare the effects of androgen and estrogen on the growth of cysts in castrated hosts. Castration removes the major source of sex hormones though the adrenal cortices may produce small amounts, thus the effects of the administered hormones and not those of endogenous hormones could be studied.

Experiment 5. Sixty, 3½ month-old female $LD_1F_1$ were used for the experiment and were divided into 4 groups.

Group I - 15 intact, infected controls.

Group II - 15 castrated, infected controls.

Group III - 15 castrated, infected, injected with testosterone.

Group IV - 15 castrated, infected and injected with estradiol.

Mice were infected with a 40 per cent cyst suspension obtained from the 64th cotton rat transfer. Hormone treatment began the day following infection. The dose of testosterone propionate was 8.0 µg/g, that of estradiol benzoate, 4.0 µg/g. The injections were given twice a week

TABLE XIV

Growth of Larval E. multilocularis in Hormone-treated, Castrated Female $LD_1F_1$ Mice

| Group[a] | Castration (days before infection) | Cyst Conc. % | Number Autopsied | Autopsy DPI[b] | Cyst Weight | | |
|---|---|---|---|---|---|---|---|
| | | | | | gm. | t | P |
| I intact control | - | 40 | 15 | 42 | $3.25 \pm 0.42$ | | |
| II castrated control | 7 | 40 | 15 | 42 | $1.40 \pm 0.38$ | I vs II 3.27 | $< .01$ |
| III castrated + testosterone | 7 | 40 | 15 | 42 | $2.85 \pm 0.58$ | I vs III 0.56 | $\geqslant .05$ |
| IV castrated + estradiol | 7 | 40 | 14 | 42 | $3.06 \pm 0.43$ | I vs IV 0.32 | $> .05$ |

[a] 3 1/2 months old at infection

[b] Days post infection

TABLE XV

Host Responses of Hormone-treated, Castrated Female $LD_1F_1$ Mice

| Group | Weight at Infection gm. | Net Weight[a] at Autopsy gm. | Weight[b] Change gm. | Spleen Weight gm. | Kidney[c] Weight gm. | Ovaries[d] Weight gm. | Uterus[e] Weight gm. |
|---|---|---|---|---|---|---|---|
| I intact control | 20.00 | 23.05 | +3.05 | $0.47^{+}_{-}$ 0.04 | $0.24^{+}_{-}$ 0.008 | $0.06^{+}_{-}$ 0.004 | $0.15^{+}_{-}$ 0.010 |
| II castrated control | 22.13 | 24.80 | +2.67 | $0.29^{+}_{-}$ 0.04 | $0.20^{+}_{-}$ 0.003 | - | $0.11^{+}_{-}$ 0.008 |
| III castrated + testosterone | 21.60 | 25.22 | +3.62 | $0.36^{+}_{-}$ 0.04 | $0.34^{+}_{-}$ 0.011 | - | $0.25^{+}_{-}$ 0.014 |
| IV castrated + estradiol | 21.73 | 23.19 | +1.46 | $0.33^{+}_{-}$ 0.02 | $0.25^{+}_{-}$ 0.005 | - | $0.25^{+}_{-}$ 0.011 |

[a] Net weight = Gross weight - Cyst weight

[b] Weight change = Net weight at autopsy - Body weight at infection

[c] Right kidney

[d] Both ovaries

[e] Both horns of uterus

for 5 weeks. Autopsy was performed 42 DPI. The results are summarized in Table XIV.

Cyst weights: Both castration and hormone treatment affected the size of cysts (Fig. 17). Growth of cysts in the castrated controls was significantly slower than that in the intact controls ($P < .01$). In testosterone and estradiol-treated mice the cyst size was basically the same as in the intact controls. Estradiol benzoate-treated mice had slightly heavier cysts than those treated with testosterone propionate, but the difference was not significant. Protoscoleces were observed in both the testosterone and estradiol treated groups.

Host reactions are summarized in Table XV. One death occurred in Group IV prior to termination of the experiment. The mean gross body weight increased in all groups. A steady increase was observed in the intact controls (Fig. 18). In the castrated controls and estradiol-treated group an increase of over 3 gm. occurred during the first week of infection with very little increase after this time. Testosterone-treated mice gained in weight considerably in the course of the experiment, the average per mouse being over 7 gm. Net weight increased were observed in all groups, the largest in Group I and III (Table XV).

Spleen enlargement was present in all groups. Castration decreased the kidney weights while testosterone treatment increased them above the normal 0.24 gm. Estradiol treatment returned it to normal. Uterine weight was depressed in the castrated controls and was above normal in the testosterone and estradiol-treated castrated groups.

Figure 17. Growth of cysts in hormone-treated castrated mice. Experiment 5.

Numbers over columns indicate number of mice autopsied.

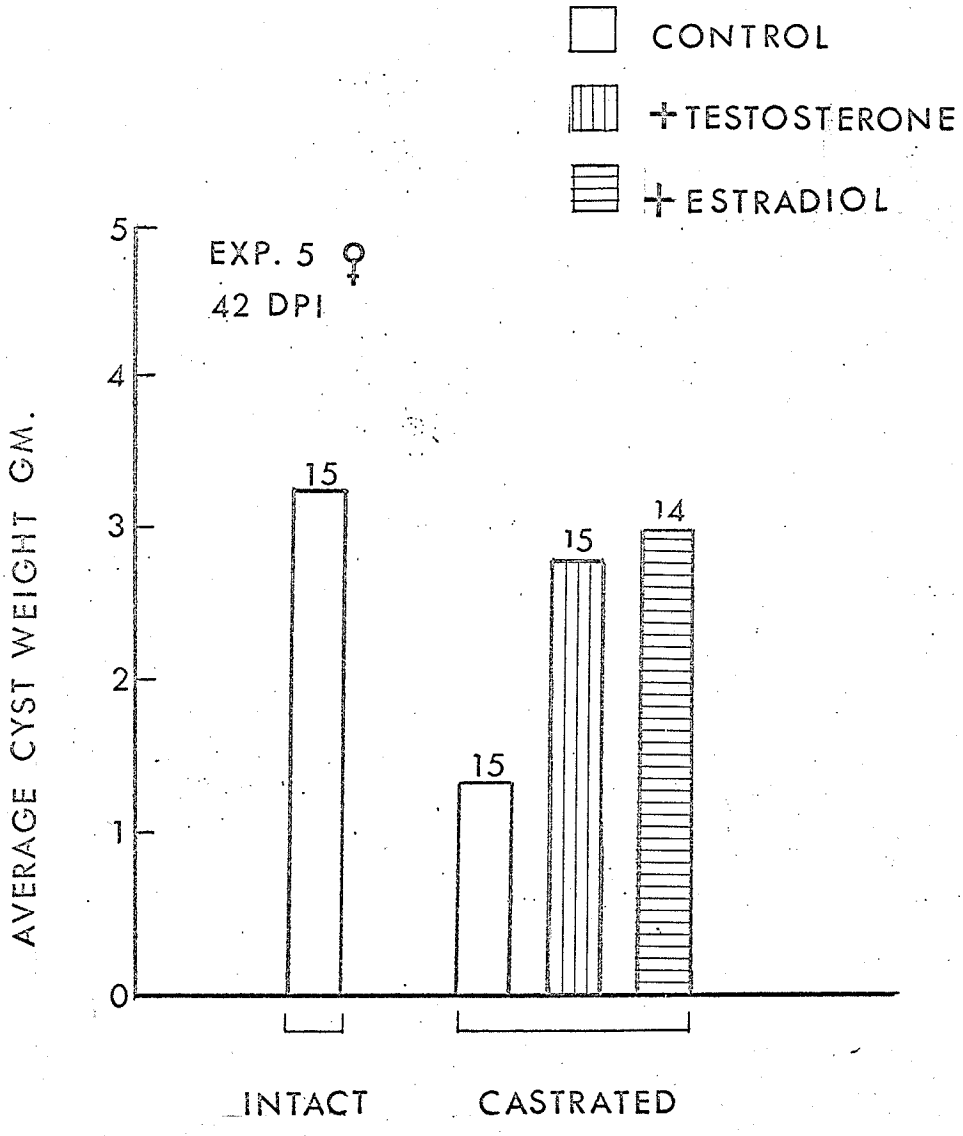
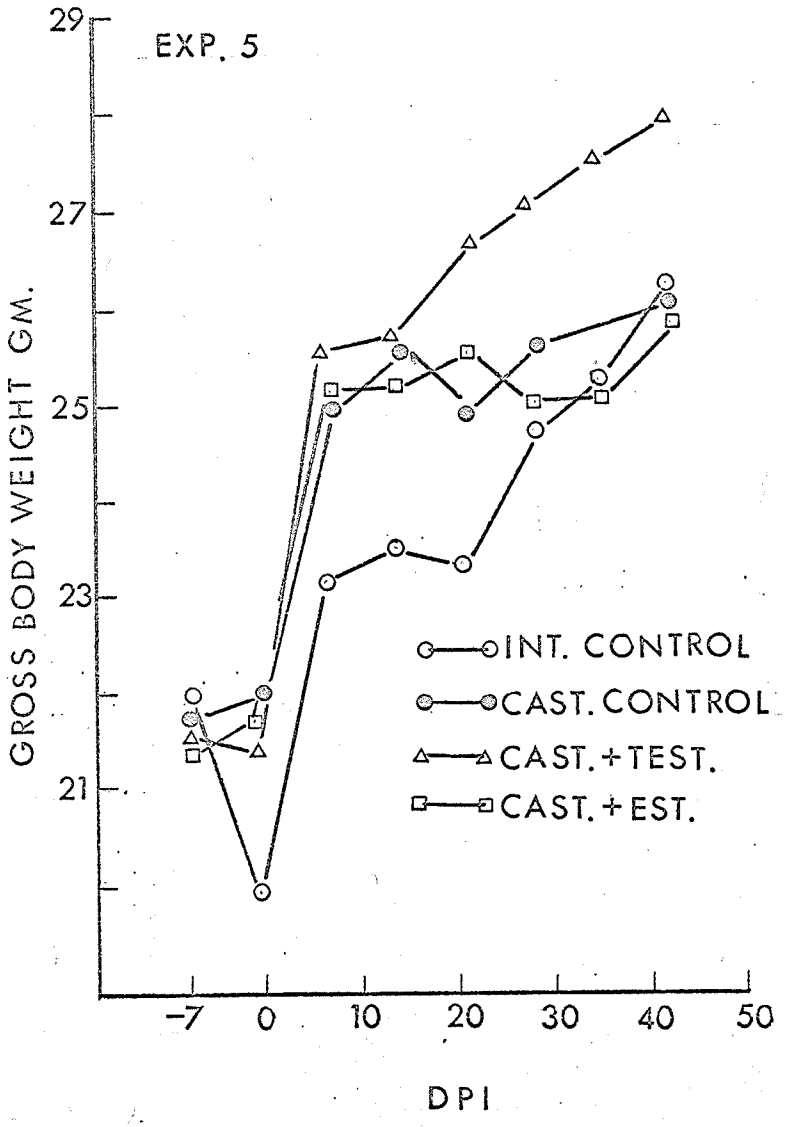

CONTROL
+TESTOSTERONE
+ESTRADIOL
EXP. 5 ♀
42 DPI
AVERAGE CYST WEIGHT GM.
0
1
2
3
4
5
15
15
15
14
INTACT
CASTRATED

Figure 18. Gross body weight changes in hormone-treated, castrated mice. Experiment 5.


EXP. 5
GROSS BODY WEIGHT GM.
29
27
25
23
21
-7
0
10
20
30
40
50
DPI
INT. CONTROL
CAST. CONTROL
CAST. + TEST.
CAST. + EST.

# DISCUSSION

## Sex of the Host and Growth of E. multilocularis

The sex of the host does influence the course of many helminthic infections. This is true in the case of both adult and larval helminths. Both the susceptibility of the host and/or the growth rate of parasite are influenced by the sex of the host.

In some cases the male is the better host while for other helminths the female is better. The parasites which are influenced by host sex are listed in the review of literature (Table I).

My experiments with E. multilocularis confirmed the results of Lubinsky (1967). In the female host, cyst growth was greater than in the male host. This observation was extended to SWR and SEC mice and gerbils, but the differences were statistically significant only in SWR mice. The fact that gerbils had the highest ratio of cyst weight to net body weight indicates that they are good experimental hosts. Individual variability in cyst growth is as large in these hosts as in mice so that large numbers of gerbils would have to be used. These were not available in this study.

The faster growth in the females can possibly be attributed to the gonadal hormones and be interpreted to mean that the female provides a more favourable milieu for the growth of E. multilocularis. However, the effects of castration and hormone treatment must be determined before this can be proved.

## Castration of the Host and Its Effects on E. multilocularis

Castration of the host significantly changes the course of many helminthic infections. It may increase the susceptibility of the host

or vice versa. The effects of host castration on helminths are summarized in Table II.

My experiments on the effects of host castration show that:

1. Castration results in slower growth of the cysts.

2. Cysts grow slower in castrated hosts regardless of the host's sex or age.

3. The period of initial slow growth of cysts in castrated hosts is extended substantially over that in the controls (Fig. 11).

There appear to be 2 ways in which castration may alter the growth pattern of the larvae. First, indirectly, by altering the metabolism of the host; and second, directly, by inhibiting or stimulating the metabolic processes of the parasite.

Castration obviously affects the host's metabolism in that protein synthesis is decreased (Turner 1966; Engel 1941). Apparently there is a decrease in transaminase activity (Awarpara and Seale 1952) in several tissues of the castrated rodent which ultimately results in the slow down of protein synthesis. This slow down may indirectly alter the growth rate of the parasite.

If castration of the host were affecting the parasite directly, one could assume that gonadal hormones were necessary for the development of the cestode. Hormones control metabolic processes through action at the enzyme level of activity (Turner 1966) though their exact role is unknown. Aldrich et al. (1954) found that transaminase activity was reduced in the tissues of tapeworms from castrated hosts. As a result there was increased fat deposition, explained as an increased channelling of Krebs cycle constituents toward fat as a result of inhibition of protein synthesis. Daugherty (1956) found that castration of the rat host caused a decline in the rate of glycogen synthesis from both glucose and pyruvate

in Hymenolepis diminuta. He believed that these changes were manifestations of a general reduction in the level of metabolism. Wertheim et al. (1960) found that cestodes could not carry out transamination to a great extent and that they probably absorb the required amino acids from the surrounding medium.

Daugherty (1956) stated as an alternative theory that castration affects the physiology of Hymenolepis by changing the host and these changes should not be attributed to any direct effect of the absence of hormones on the parasite. The fact that cestodes may be successfully cultured in vitro without the presence of sex hormones (Schiller 1965; Smyth 1969) strengthens the hypothesis that the lack of sex hormones does not directly affect the growth of the parasite.

In my experiments on the effects of host castration, the growth of cysts in castrated hosts was significantly slower than in the controls, regardless of sex. In the males the differences were statistically significant in all 4 experiments with cyst growth being at most one-half that in the controls. The differences in the females were not as large, and were statistically significant only in Experiments 3 and 4 (Table VII) (Fig. 6).

It is known that androgen promotes protein synthesis by maintaining a positive nitrogen balance whereas estrogen affects protein metabolism but slightly (Guyton 1966). Thus castration of the male decreases anabolism and consequently decreases growth. This was observed in my experiments on castration and is demonstrated by decreases in weight gains of castrated males as compared to the controls (Table VIII) (Figs. 7, 8, 9, 10). Cysts grow slower in castrated males as already mentioned. On the

basis of these results one can correlate host metabolism with cyst growth and state that the growth of cysts is dependent on the host's metabolism.

In the castrated females cyst weights were diminished but not to as great an extent as in the males. Metabolism in the female is not depressed greatly by castration and may increase slightly. This fact may explain why cyst growth in castrated females is not affected as greatly as in the castrated males.

To clarify the situation, mice of both sexes were infected 30 days after castration. This would make certain that no circulating sex hormone produced by the gonads was present in the host. In the males the results were essentially the same as in the experiments in which mice were infected 7 days after castration but in the females the depression of cyst growth was greater and closer to that in castrated males. This may indicate that the metabolism of the female changes after castration more slowly than that of the male. It is also possible that circulating estrogen may have been affecting cyst growth for some time after castration in females infected 7 days after castration.

From the growth curve of cysts in Experiment 5, it is evident that the period of initial slow growth is substantially longer in castrated hosts than in the controls (Fig. 11) and that the cysts continue growing at a reduced rate. Therefore it is probable that cyst growth is not directly dependent on sex hormones but affected indirectly by a slow down of the host's metabolism.

One may argue that sham-operated controls were not used, however in a study of the effects of splenectomy, Lee (1970) found no differences in cyst growth between these and unoperated controls.

The Influence of Exogenous Sex Hormones on E. multilocularis

My experiments demonstrated that sex hormones affect the growth of E. multilocularis in $LD_1F_1$ mice.

Testosterone propionate tends to increase the growth rate of cysts both in male and female mice. The intact females in Experiment 1, and the intact males in Experiment 2, treated with testosterone had significantly greater cysts than the controls (Fig. 13). This was also true in Experiments 3 and 4 but the differences in cyst weights between control and treated mice were smaller. Testosterone also accelerated the growth of cysts in castrated males in Experiment 2 and 4.

In the review of literature it was pointed out that testosterone and other androgens affect the susceptibility of a wide range of animals to various parasites. Studies with cestodes support the present findings that the growth of parasites in hosts treated with high doses of testosterone is greater than that in controls. This is true in respect of both intact and gonadectomized hosts as was shown by Campbell (1939) and Campbell and Melcher (1940) with Taenia taeniaeformis; Addis (1946) and Beck (1952) with Hymenolepis diminuta; and Meyer and Valleau (1967) with Diphyllobothrium sebago.

Both similar and conflicting results were obtained in respect of nematodes. Heavy doses of testosterone increased the susceptibility of very young chicks to Ascaridia galli whereas low to moderate doses decreased it (Sadun 1951). Todd and Crowdus (1951) found that methyl testosterone in the diet of chickens did not affect the number of A. galli but the worms were longer in the treated group. Berg (1957) found that testosterone injections decreased the number of female

Schistosoma mansoni recovered from castrated males, while Mathies (1959b) found that it did not affect worm burdens in either male or female mice infected with Aspicularis tetraptera.

Estradiol benzoate also tends to increase the growth rate of cysts in male and female mice. The intact females in Experiment 1, and the intact males in Experiment 2, had significantly greater cyst weights than the controls. In Experiments 3 and 4 there were also differences between growth in treated and control mice but they were not statistically significant (Table XII).

In Experiments 1 and 3 weight of cysts from estradiol-treated castrated females were close to those from intact controls. In both experiments the cysts in castrated controls were significantly smaller than in castrated and estradiol-treated animals. Chorionic gonadotrophin had no effect on cyst growth in either intact male or female mice.

The influence of female gonadal hormones on the growth of various parasites was studied by many authors. In some cases estrogen injections lowered the parasite load. This was found in male rats infected with Taenia taeniaeformis by Campbell (1939) and Campbell and Melcher (1940); in young female chickens infected with Acaridia galli by Ackert and Dewhirst (1950), and Sadun (1951); in male mice infected with Aspicularis tetraptera by Mathies (1959b); in naturally infected frogs by Lees and Bass (1960; and in castrated male and female rats infected with Nematospiroides dubius by Dobson (1961b).

In some cases estrogen did not affect nematode infections. This was observed by Solomon (1966) in rats infected with Nippostrongylus brasiliensis and in rabbits infected with Trichostrongylus axei and T. colubriformis by Rohrbacher (1958 and 1960).

The effects of progesterone on helminthic infections have been studied as well. Progesterone is just as effective in increasing susceptibility to infections as testosterone (Addis 1946; Beck 1952; and Solomon 1966). It is interesting to note that the pathway of degradation of progesterone via pregnanediol and pregnanetriol may be substituted by a pathway in which testosterone is formed (Villee 1961).

My last experiment on cyst growth in hormone-treated mice was designed to compare the effects of testosterone propionate on the growth of cysts in castrated mice with that of estradiol benzoate. The growth of cysts both in testosterone and estradiol-treated mice was comparable to that in the intact controls even though the dose of estradiol was half as large as that of testosterone. It seems that both hormones accelerate cyst growth in castrated mice. In no other host-parasite system has it been shown that both androgen and estrogen increase the growth of parasites.

Haley (1958) working with Nippostrongylus muris in hamsters , Mathies (1959b), with Aspicularis tetraptera in mice and Dobson (1961a,b), with Nematospiroides dubius in mice and rats attributed differences in susceptibility and growth of parasites between the sexes to endocrinological changes occurring during sexual maturation. Mathies (1954, 1959a,b) stated that estrogens changed the susceptibility of mice to A. tetraptera. Dobson (1961a) suggested direct and indirect mechanisma for the estrogen control of the development of Nematospiroides dubius in the mouse. He (1961b) stated that:

> "it is evident that the male host is more susceptible to infection than the female; estrogen tending to decrease the susceptibility while testosterone has little or no effect."

It is interesting to note that estrogen stimulates the phagocytic activity of the RES (Heller et al. 1957).

Some authors believe that gonadectomy and gonadal hormones influence the development of the integument of the host and this alters the susceptibility of the host (Haley 1958; Solomon 1966). This may be true in the case of some skin penetrating parasites but cannot be applied to E. multilocularis.

It is possible that the growth of E. multilocularis may be sex-dependent as is tumor growth since both are stimulated by estrogen. However, cyst growth is also accelerated by testosterone whereas tumor growth is inhibited by androgens.

Although no definite answer to the question of the role of host sex in the development of E. multilocularis has been found, it is certain that it does have either a direct or indirect influence on cyst growth. A system in which a direct influence has been postulated is the rabbit-rabbit flea system (Rothschild and Forb 1966). Here the parasites' reproduction is geared to that of the host's by the host's sex hormones. It is believed that the mammalian hormones regulate the release of the fleas' own sex hormones. Therefore, even in this close association the host's hormones do not directly affect the development of the parasite.

The fact that larvae of E. granulosus will develop to the adult stage in vitro if provided with a solid substrate, but will form hydatid cysts if this is not provided (Smyth 1967) indicates that this cestode and probably E. multilocularis as well, cannot have too complex an endocrinological system. It is unlikely that the larvae are utilizing mammalian sex hormones directly, but these hormones may trigger the

cestode's endocrinological system. It is more likely that sex hormones influence the host's metabolism and affect the parasite indirectly.

Age of the Host and E. multilocularis

There are many references in the literature to a greater resistance of older animals against establishment of parasites. This was observed in Ancylostoma duodenale and A. caninum in the dog (Sandground 1929); Ascaridia lineata and Syngamus trachea in the chicken (Sandground 1929); Ascaridia galli in the chicken (Ackert et al. 1939); Aspicularis tetraptera in the mouse (Mathies 1959a; Stahl 1961); Litomosoides carinii in the rat (Olsen 1959); Strongyloides ratti in the rat (Sheldon 1937); Haemonchus placei, Ostertagia ostertagia, Trichostrongylus axei, T. colubriformis, Cooperia punctata, C. pectinata, Nematodirus helvetianus and Oesophagostomum radiatum in cattle (Herlich 1960).

Schwabe et al. (1959) found that the growth of Echinococcus granulosus was faster in mice 48 days old or younger than in mice 71 days or older. According to my observations male $LD_1F_1$ mice exhibit age resistance in respect of E. multilocularis. In mice 1 month of age cysts grow faster than in older mice (Fig. 5).

Several theories have been suggested to account for age resistance. According to Sandground (1929) age resistance is just an extension of natural resistance. Mathies (1959a) thought that age resistance and sex resistance were linked in some way and suggested that the decrease in susceptibility to infection with increasing age was due to hormonal changes accompanying sexual maturity.

Schwabe et al. (1959) stated that host resistance to E. granulosus is dependent upon rapid destruction of protoscoleces by the host's cellular

reaction and that its intensity might be related to the sexual maturity of the host. In other words, age resistance develops with the sexual maturity of the individual. This may be the case since in my experiments with E. multilocularis mice 7 weeks of age, just reaching sexual maturity, were more heavily infected than older mice. It would be interesting to compare the growth of cysts in immature mice with that in mature mice but this was beyond the scope of the present project.

## CONCLUSIONS

1. The growth of larval E. multilocularis is faster in female gerbils, and female SEC, SWR, and $LD_1F_1$ mice than in male gerbils and mice.
2. The growth of cysts in 1-month-old mice is faster than in 6-month-old mice. After this age, the growth becomes progressively faster but even in 18-month-old mice it is still slower than that in 1-month-old mice.
3. Cysts of E. multilocularis grow slower in castrated hosts, independently of the host's sex or age.
4. The initial period of slow growth of cysts in castrated hosts is longer than that in the controls.
5. Testosterone propionate, in a dose of 8.0 $\mu$g/g X 10, and estradiol benzoate in a dose of 4.0 $\mu$g/g X 10, increase the growth rate of cysts in intact and castrated male and female mice.
6. Chorionic gonadotrophin in doses up to 1.6 I.U./g X 10, had no effect on the growth of cysts in intact male and female mice.
7. Both testosterone and estradiol accelerate the growth of cysts in castrated females. The weight of cysts in females treated with testosterone, 8.0 $\mu$g/g X 10, was the same as in females treated with estradiol, 4.0 $\mu$g/g X 10.

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