The Influence of Splenectomy and Cortisone on the Growth of Larval *Echinococcus multilocularis* Leuckart, 1863

A Thesis
Presented to
the Faculty of Graduate Studies and Research
University of Manitoba

In partial fulfillment
of the Requirement for the Degree
Master of Science

by

Chiu-Fan Lee

Spring , 1970

The influence of splenectomy and of cortisone on the growth of larval Echinococcus multilocularis Leuckart, 1863

Abstract

1. The effects of the blockade of the RES, splenectomy and cortisone on the growth of intraperitoneal Echinococcus multilocularis cysts in rodents were studied.

2. Carbon particle blockade did not affect the growth of cysts in LD_1F_1 and CBA mice.

3. Preliminary experiments with mice, hooded rats, and gerbils showed that splenectomy does not affect the growth of Echinococcus cysts.

4. The study of the growth curves of Echinococcus cysts in intact and in splenectomized mice showed that these two curves are almost identical both in experiments with LD_1F_1 and with $B6D2F_1$ mice.

5. Cortisone accelerated the growth of Echinococcus cysts in LD_1F_1 mice of both sexes, in shortening the initial phase of slow growth from over 40 days to about 20 days. The greatest difference in cyst weight between treated mice and the controls occurred between the 44th and 57th day after infection.

6. Early in the course of Echinococcus infections, LD_1F_1 mice developed a very high leucocytosis with pronounced neutrophilia, but without lymphocytosis. Cortisone lowered the number of lymphocytes and monocytes, but had no effect on the absolute neutrophil count.

7. In LD_1F_1 mice the growth curve of intraperitoneal cysts is sigmoid and the mice die in the period of fast growth of cysts.

8. In $B6D2F_1$ mice the growth curve is also sigmoid, but the phase of fast growth is followed by a decline in cyst weight.

9. Of the three experimental approaches used, the blockade of RES, the splenectomy, and the cortisone treatment, only the last has produced positive results, namely a considerable acceleration of cyst growth in the early stages of infection.

ACKNOWLEDGEMENTS

I thank my advisor, Dr. G. Lubinsky, for the proposed topics of research, his guidance in the process of my work and the generous supply of experimental animals.

I sincerely appreciate the valuable constructive criticism given me by Dr. H. E. Welch. I also thank him for his kind permission to work in the Department of Zoology.

Special thanks are due to Mrs. Katherine Friesen for her encouragement during the course of the work, to Mr. Robert Baron for technical assistance, to Dr. P. H. Wong, Mr. Frederick Austin and Mr. Philip Ould for their many inspiring suggestions and to Mrs. H. Schwanke for her patient and careful work in typing this thesis.

CONTENTS

INTRODUCTION.....1

REVIEW OF LITERATURE
1. Echinococcus multilocularis Leuckart, 1863..... 3
   a. The genus Echinococcus
   b. Taxonomy & description of E. multilocularis
   c. History & distribution
   d. Life cycle
   e. Growth of cysts in laboratory mice
   f. Factors affecting the growth in laboratory mice
2. The reticuloendothelial system..... 16
   a. History and definition
   b. Structure of the RES
   c. Functions of the RES
   d. Experimentation on RES activities
3. Indian ink blockade.....25
   a. Effects on the host
   b. Parasitic infections
4. Splenectomy.....29
   a. The spleen
   b. Effects of splenectomy on the host
   c. Effects on parasitic infections
5. Cortisone treatment.....40
   a. Cortisone
   b. Effects on the host
   c. Effects on parasitic infections

MATERIAL AND METHODS
1. Parasite and hosts.....50
2. Infection with vegetative propagation of larval Echinococcus multilocularis.....51
3. Carbon particle blockade.....54
4. Splenectomy.....55
5. Cortisone treatment.....58
6. Haematological techniques.....58
7. Photography.....60

PLANS OF INVESTIGATION.....60

RESULTS
1. The blockade of the RES with Indian ink.....61
2. Splenectomy.....63
   a. Observations on various rodents.....64
   b. Protracted experiments with susceptible LD_1F_1 mice.....66
   c. Protracted experiments with more resistant male $B6D2F_1$ mice.....76

3. Cortisone & the growth of Echinococcus cysts 84
a. Drug tolerance test 85
b. Preliminary experiment with cortone (Experiment I) 85
c. Protracted cortone experiment (Experiment II) 87

DISCUSSION
1. Some sources of experimental errors 101
2. Evaluation of the experimental results 101
a. Indian ink blockade of the RES 102
b. Splenectomy 104
c. Cortisone and the growth of Echinococcus multilocularis cysts 107

CONCLUSION 112

SUMMARY 113

REFERENCE 115

APPENDIX I: Glossary 130

APPENDIX II: Chemotherapy of larval Echinococcus multilocularis in LD_1F_1 mice 131

APPENDIX III: Correlation of spleen weight with the weight of Echinococcus cysts in six genetically different types of female mice 132

List of Figures

1. Transmission of Echinococcus multilocularis.....2

2. Life cycle of Echinococcus multilocularis in red fox and cotton rat.....10

3. Effect of mouse strain on the growth of larval Echinococcus multilocularis.....14

4. The spleen.....30

5. Cortisone.....41

6. Infection apparatus of rodents with a vegetatively propagated strain of Echinococcus multilocularis.....53

7. Plastic rodent immobilizer for intravenous injection and collection of peripheral blood from the caudal lateral veins.....53

8. Technique of splenectomy.....56

9. Growth of larval Echinococcus multilocularis in LD_1F_1 male mice splenectomized 3 days prior to infection.....70

10. Mortality rate of splenectomized LD_1F_1 male mice infected with Echinococcus multilocularis.....71

11. Host responses in splenectomized LD_1F_1 male mice infected with Echinococcus multilocularis 72

12. Splenomegaly in mice infected with Echinococcus cysts.....73

13. Growth of larval Echinococcus multilocularis in $B6D2F_1$ male mice splenectomized 2 days prior to infection.....78

14. Mortality rate of splenectomized $B6D2F_1$ male mice infected with Echinococcus multilocularis.....81

15. Host responses in splenectomized $B6D2F_1$ male mice.....83

16. Growth of larval Echinococcus multilocularis in cortisone treated LD_1F_1 male mice.....91

17. Mortality rate of cortisone treated LD_1F_1 male mice infected with Echinococcus multilocularis.....93

18. General host responses in cortisone treated LD_1F_1 male mice.....94

19. Leucocyte responses in cortisone treated LD_1F_1 male mice infected with Echinococcus multilocularis.....99

List of Tables

I. Effects of splenectomy on the course of plasmodial and babesial infections of mammals and birds (Todorovic et al 1967) 35

II. Growth of larval Echinococcus multilocularis in ink-blockaded male mice.....62

III Growth of larval Echinococcus multilocularis in splenectomized rodents.....65

IV. Growth of larval Echinococcus multilocularis in splenectomized LD_1F_1 mice.....67

V. Host responses of splenectomized LD_1F_1 mice.....68

VI. Growth of larval Echinococcus multilocularis in splenectomized $B6D2F_1$ male mice.....77

VII Host responses of splenectomized $B6D2F_1$ male mice.....79

VIII Growth of larval Echinococcus multilocularis in cortisone treated LD_1F_1 mice.....86

IX Host responses of cortisone treated LD_1F_1 male mice infected with Echinococcus multilocularis.....89

X Absolute leucocyte counts in cortisone treated LD_1F_1 male mice.....96

XI Differential leucocyte counts in cortisone treated LD_1F_1 male mice.....97

INTRODUCTION

Larval Echinococcus multilocularis is the etiological agent of human alveolar hydatid disease. It is endemic in sylvatic or rural canine-rodent cycle in Europe, Asia and North America. Man is an accidental intermediate host of this cestode (Fig. 1). Community sanitary measures and surgery have been used to control this dangerous disease in humans.

The natural defensive mechanisms of the intermediate host against this parasite remain to be investigated. Failure of this larval cestode to establish itself in an intermediate host may result from two causes, namely; (1) unfavorable internal environment of the host, and (2) efficient host defenses. In the present study, some defense reactions of the host associated with the reticuloendothelial system are investigated. Three experimental approaches were used in this project. They are: (1) inhibition of phagocytosis by colloidal carbon particles; (2) splenectomy and (3) antiphlogistic effects of cortisone treatment.

The basic biological tool of the present research is a special hybrid of Jackson Laboratory mice, LD_1F_1 (C57L female X DBA_1 male), developed by Dr. G. Lubinsky (Fig. 3). In these hybrids, the intraperitoneal Echinococcus cysts grow extremely fast. Other rodents in which the growth of the cysts is sometimes slow, were used whenever necessary.

The experimental data obtained in this study should be of use in the development of the therapy of the hydatid disease.

Figure 1

Transmission of *Echinococcus multilocularis*

Based on Schiller (1954), and

Leiby & Nickel (1968),

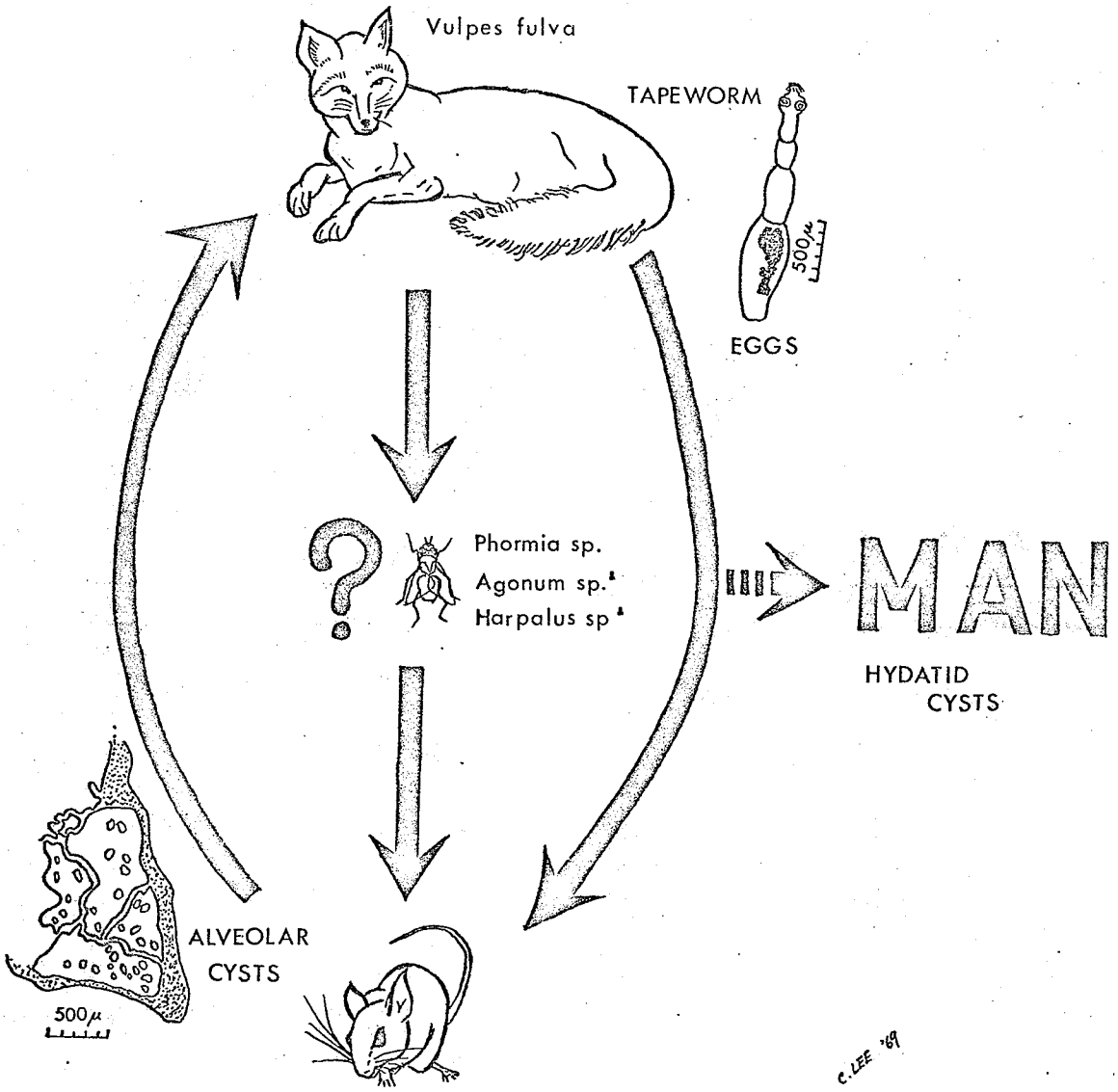


FIGURE / TRANSMISSION OF *Echinococcus multilocularis*

*Beetle grubs

REVIEW OF LITERATURE

1. *Echinococcus multilocularis* Leuckart, 1863

a. The genus Echinococcus

The genus *Echinococcus* belongs to the family Taeniidae. Species of this genus cause hydatid disease (synonyms: hydatidosis, hydatosis, echinococcosis or echinococciasis) which is characterized by the presence of cystic larvae in the tissues of the intermediate host. Smyth (1964 and 1969) reviewed extensively the biology of the hydatid organisms. Rausch (1968) recognized only 4 valid species of *Echinococcus*, namely:

E. granulosus Batsch, 1786,
E. multilocularis Leuckart, 1863,
E. oligarthrus Diesing, 1863, and
E. patagonicus Szidat, 1960.

In brief, *E. granulosus* is maintained basically in a canine-ungulate cycle and is almost cosmopolitan in distribution. *E. multilocularis* exists in a canine-rodent cycle and has been found only in the northern hemisphere. Adults of *E. oligarthrus* were reported from *Felis concolor* and *F. yagouaroundi* in Brazil and Panama (Thatcher and Sousa, 1967); their intermediate hosts are not known. Adults of *E. patagonicus* in the Magellan fox (*Dusicyon culpaeus culpaeus*) were reported from Argentina, but its intermediate hosts are also not known.

My review is primarily concerned with larval *Echinococcus multilocularis*. This species is the etiological agent of human alveolar hydatid disease.

b. Taxonomy and Description of Echinococcus multilocularis

Phylum	Platyhelminthes
Class	Cestoidea
Subclass	Eucestoidea
Order	Cyclophyllidaea
Family	Taeniidae
Genus	Echinococcus
Species	multilocularis Leuckart, 1863

Echinococcus multilocularis Leuckart, 1863 (synonyms: Echinococcus alveolaris Klemm, 1883, Alveococcus multilocularis (Leuckart, 1863) Abuladze, 1959, Taenia echinococcus Batsch, 1786 (pro parte) and Echinococcus sibiricensis Rausch & Schiller, 1954) is a cyclophyllidean cestode.

E. multilocularis is a polyzoic tapeworm that matures in carnivorous hosts. The basic morphological features are as follows (Rausch, 1956; Smyth, 1964; Verster, 1965):

size of strobila	1.2 to 3.7 mm.
number of segments	3 to 5
testes	12 to 31
distribution of testes	0 to 6 anterior to genital pore
position of genital pores	in the anterior third of both mature and gravid segment
rostellar hooks	12 to 34
size of hooks	22 to 34 µ
size of embryophore	29.5 µ to 40.5 µ by 27.5 µ to 39.5 µ

Microtine rodents are the principal intermediate hosts in which an onchosphere develops into alveolar cysts. A typical hydatid cyst is composed of a double-layered wall (germinal membrane and laminated membrane), protoscoleces and hydatid fluid. Each protoscolex has 11 to 36 hooks. The larvae grow by successive exogenous and endogenous budding (Fig. 2).

Valid sub-species are E. m. multilocularis Vogel, 1955 and E. m. sibiricensis Rausch & Schiller, 1954.

The chromosome number of the cells of the germinal membrane is 18 (Sakamoto et al 1967, after Smyth, 1969).

C. History and Distribution

Echinococcus multilocularis appears to be limited to the northern hemisphere. The natural endemic areas are Central Europe, Siberia, North Pacific Islands and North America.

The alveolar cysts were first recognized as Echinococcus cysts by Virchow in 1855. He showed that a peculiar pathological condition of human liver, previously believed to be a mucoid or colloidal carcinoma was in reality a larval stage of the tapeworm Echinococcus. Since then, the disease has been diagnosed frequently in southern Europe, Russia and some other countries. Leuckart (1863) designated E. multilocularis as alveolar cysts differing from those of "Taenia echinococcus". Barabash-Nikiforov (1938) recorded alveolar larvae of "Taenia echynococcus" in almost 50% of Clethrionomys rutilus from the Bering Island in the Bering Sea. The alveolar cysts in the tundra vole, Microtus oeconomus was found in 1950 on St. Lawrence Island, Alaska (Rausch and Schiller, 1951). Later adult cestodes were found in the arctic fox, Alopex lagopus and the red fox on the Alaskan mainland. The new species was described as Echinococcus sibiricensis by Rausch and Schiller (1954). Inukai, Yamashita and Mori (1955) reported E. multilocularis in Vulpes fulva from the Japanese Island of Rebun; these workers suggested that the cestode was introduced to Rebun Island with red

foxes from Kurile Islands, to which it was brought from Kamanodorskii Islands. Human infections on Rebun Island were first noted about 10 years after the introduction of the red foxes. Several cases of human infections were found in Eskimos in Alaska. Man may be infected with onchospheres as a result of contact with sled dogs (Rausch, 1956). At that time the identity of the cestode causing alveolar hydatid in man was a matter of controversy. Vogel (1957) repudiated, on the basis of experiments, the views of some helminthologists such as Deve and Dew that E. multilocularis was an abnormal E. granulosus. Confirming Posselt's dualist view, Vogel established two statements: (1) E. multilocularis is a species distinct from E. granulosus and (2) the tapeworms of E. multilocularis from Southern Germany and those of E. sibiricensis Rausch & Schiller, 1954, from Alaska morphologically resemble each other to such an extent that they must be regarded as one species. Rausch (1956) reported no natural infections in approximately 2500 rodents examined on the Alaskan mainland in 1949-1955. During the same period on St. Lawrence Island, the rate of infection in Microtus which never exceeded 20%, was sufficient to achieve 100% infection in the Arctic foxes. Rausch further postulated that E. multilocularis will be introduced into the continental United States and southern Canada by dogs imported from Arctic regions because of the abundance of susceptible microtine rodents and large number of red foxes in the agricultural regions. E. multilocularis, either in the larvae or adult form, was found in North Dakota, Minnesota, Saskatchewan and Manitoba (Leiby and Olsen, 1964; Leiby, 1965; Hnatuik, 1966; Leiby et al, 1969; Lee, 1969). The finding of E. multilocularis in the

arctic fox from Eskimo Point on the western shore of Hudson Bay, Northwest Territories, is the first record of this cestod in Canada (Choquette et al, 1962).

In North America, only a few cases of fatal human alveolar hydatid disease have been published. The first case was that of a fisherman from Brandon, Manitoba, who came to Canada from Iceland when seven years old and died from this disease in Winnipeg in 1927 when 54 years old (James and Boyd, 1937). Biologists like West, Rausch and Leiby suggested, on the basis of recent findings of E. multilocularis on the North American continent that these human cases were autochthonous (Leiby et al, 1969). Schiller (1965) mentioned two more recent fatal cases, one reported from Edmonton, Canada, and one from Milwaukee, Wisconsin, U.S.A. The patients were born and lived in the western Ukraine before emigrating to North America. Schiller said that the original authors thought that these patients had acquired the disease before coming to North America.

d. Life Cycle

In nature, foxes, dogs, cats (Vogel, 1957), wolves and coyotes serve as definitive hosts of E. multilocularis. Microtine rodents, especialy voles, serve as principal intermediate hosts. Transmission is accomplished through predation. Man is an accidental intermediate host which becomes infected by ingesting Echinococcus eggs. Various insects such as blowflies and beetle grubs may play a secondary role as vectors transferring the onchosphere from the definitive hosts to the rodents (Schiller, 1954b; Leiby and Nickel, 1968). The parasite may be maintained in 2 ways: (1) a sylvatic cycle, e.g. between the tundra vole and the arctic fox or wolves, and (2) a rural cycle, e.g. between the deer mouse and the red fox or dogs (Fig. 1).

The cat* may serve as a definitive host (Vogel, 1957).

A list of natural and experimental intermediate hosts of *E. multilocularis* is given below (after Smyth and Smyth, 1964). Natural, primary (experimental) and secondary (parenteral) infections are indicated by N, P, and S respectively.

Order Insectivora	
Sorex jacksonii	N
Sorex tundrensis	N
Order Primates	
Macaca sp.	P
Homo sapiens	N
Order Lagomorpha	
Oryctolagus cuniculus	S
Order Rodentia	
Citellus undulatus	N
Eutamias asiaticus	P
Peromyscus gossypinus	P
P. maniculatus	N
P. truei	P
Sigmodon hispidus	PS
Cricetus barabensis	N
Mesocricetus auratus	S
Lemmus sibiricus	P
Clethrionomys rutilus	NP
Clethrionomys rufocanus	P
Ondatra zibethica	NP
Microtus oeconomus	NP
M. pennsylvanicus	NP
M. agrestis	N
M. arvalis	NP
M. gredalis	N
M. montebelli	P
M. californicus	P
Lagurus lagurus	P
Meriones unguiculatus	PS
Rhombomys opimus	N
Apodemus geisha	P
A. agrarius	N
Rattus norvegicus	P
Mus musculus	PS
Myocastor coypus	N

* Another case of tapeworms of *E. multilocularis* in the cat was reported from Saskatoon, Canada, by Globe and Mail, Toronto in February, 1970.

The report of Russian authors concerning the occurrence of E. multilocularis in cattle and sheep are almost certainly based on the misidentification of the multilocular cysts of E. granulosus.

No natural or experimental avian infections have ever been reported.

A laboratory host-parasite system is illustrated in Fig. 2. Cotton rat is highly susceptible to Echinococcus infection (Sadun et al, 1957). Serial parenteral transfers of E. multilocularis in cotton rats and gerbils provide a convenient method of maintaining the parasite without hazards involved in handling Echinococcus onchospheres which are infective to man (Lubinsky, 1960 b).

Histogenesis of larval E. multilocularis in white mice was studied by Mankau (1955 ; 1957), in various laboratory mice by Yamashita (1960), in cotton rats by Cameron (1960), and Webster and Cameron (1961) and in 19 different species of rodents by Lukashenko (1966).

The intermediate hosts of E. multilocularis can be classified into 4 groups according to their natural resistance to the infection with onchospheres (Rausch & Schiller, 1956; Webster & Cameron, 1961):

(1) Complete resistance; invasion of host tissue is never achieved.
Example: Cavia

(2) Invasion without establishment; the host tissue reaction is severe and the parasite is contained within fibrous tissue forming a pseudotubercle or granuloma.
Example: Mesocricetus auratus

(3) Invasion with slow development; inhibition due to host reaction, usually results in sterile cysts.
Example: most laboratory mice

(4) Invasion with rapid development of fertile cysts.
Example: Microtus pennsylvanicus, Peromyscus maniculatus

Figure 2

Life cycle of Echinococcus multilocularis
in red fox and cotton rat

Based on Cameron (1956), Webster & Cameron (1961), Olsen (1962) and Smyth (1964).

A. Polyzoic adult; B. embryonated eggs passed in faeces 30-35 days after infection; C. egg survival up to $2\frac{1}{2}$ years at $2^{o}C$; D. infection of intermediate host; E. oncosphere activated by bile salts; penetration of gut wall into hepatic portal circulation; F. formation of daughter cysts in liver; G. vesicularization well-developed 4 weeks after infection with onchospheres; or within 14 days after intraperitoneal infection; H. oncospheres in blood circulation; I. mature protoscoleces formed 7-8 weeks after infection with onchospheres or within 3 weeks after intraperitoneal infection with cysts; J. exogenous budding: protrusion of the germinal membrane; K. protoscolex; L. infection of definitive host; M. hydatid cysts digested and protoscoleces released; N. evagination of a scolex.

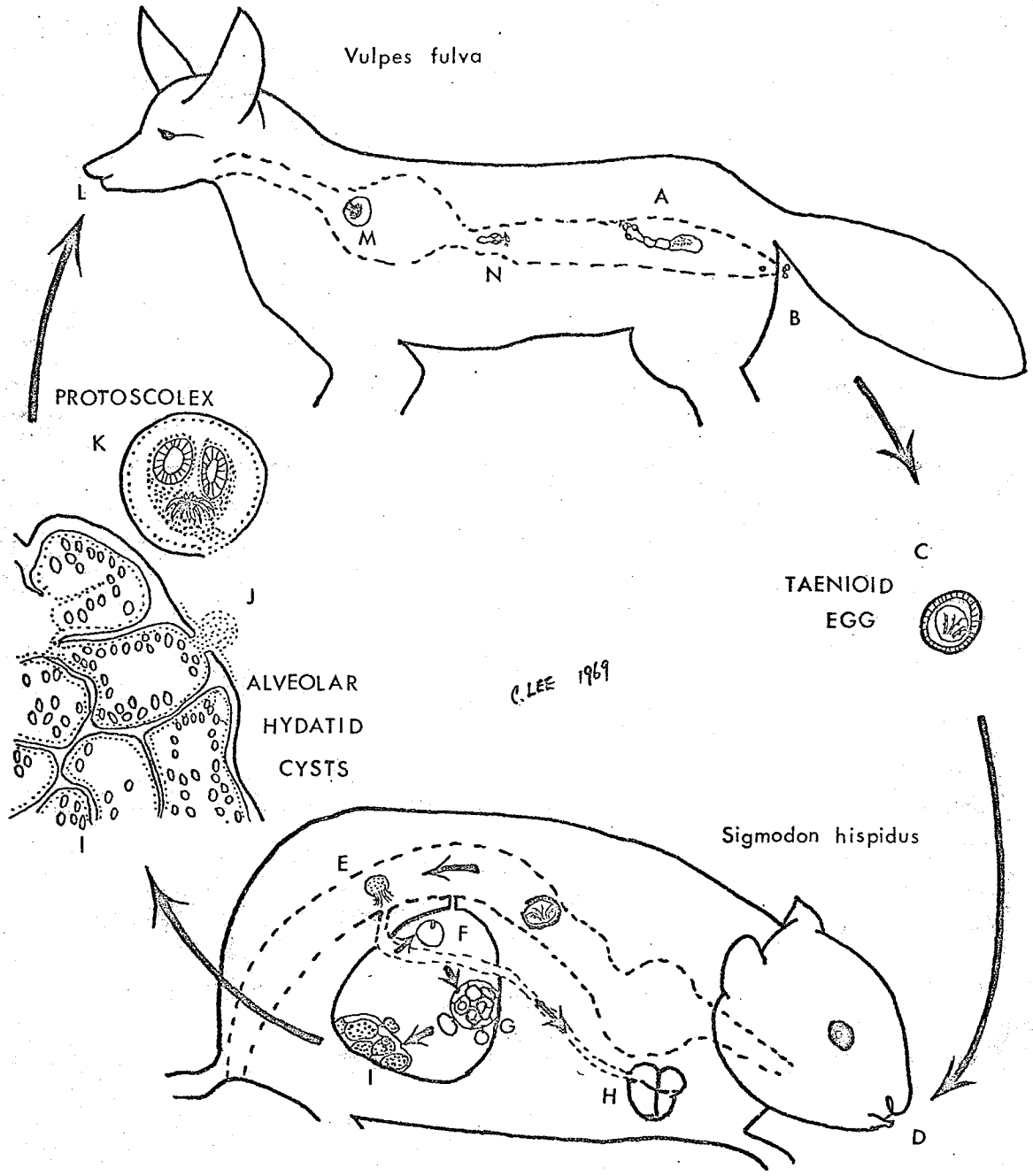


FIGURE 2 LIFE CYCLE of Echinococcus multilocularis

Details based on Webster and Cameron (1961), Smyth (1964), Cameron (1956) and Olsen (1962).

Parenteral infection with minced Echinococcus cysts may result in 2 types of growth of cysts in mice (Lubinsky):

(1) Slow growth of cysts with late production of numerous protoscoleces.
Example: $B6AF_1$ hybrid mice

(2) Rapid growth of cysts with late development of protoscoleces. These mice almost invariably die before the development of protoscoleces.
Example: LD_1F_1 mice

It is interesting to note that the development of E. multilocularis cysts in the natural hosts is very rapid. The phenomenon may be regarded as an adaptation to the short-lived hosts, e.g. Clethrionomys rutilus, Microtus pennsylvanicus and Peromyscus maniculatus.

e. Growth of Echinococcus cysts in laboratory mice

Many strains of laboratory mice, Mus musculus, are convenient experimental intermediate hosts to Echinococcus multilocularis. Primary infection (oral) with onchospheres and secondary (intraperitoneal or subcutaneous) with hydatid cysts are the two methods used. The second method enables the parasite to by-pass the host's digestive system including the hepatic portal circulation and the liver.

Tissue Reaction of the host: In chronic infections with E. multilocularis, the local host reaction is productive inflammation, leading to the development of granulomata. Webster and Cameron (1961) described the tissue reaction of the host in terms of trizonal reaction: (1) an inner layer of eosinophils, monocytes, epithelioid cells and giant cells, (2) a middle layer of fibroblasts and eosinophils and

(3) an outer fibrous layer of connective tissue which blends with the normal host tissues. The only cells which proliferate are the fibroblasts. If the host reaction is not intense, the tissue reaction becomes a simple fibrous adventitious capsule. In hosts in which the growth of Echinococcus cysts is fast, such as cotton rats, giant cells are seldom found, in contrast to human hydatid (Webster and Cameron, 1961).

Ways of infection: Mankau (1955) summarized the ways in which hydatid infections spread as follows:

(1) The embryo hatched from the egg in the small intestine is carried to the liver or other organs by the blood stream (primary infection).

(2) Protoscoleces or "other germinative parts" of the primary hydatid cysts are carried to another place by the lymph or blood stream (metastasis).

(3 Brood capsules, protoscoleces, or germinative membrane of the ruptured primary or secondary intraperitoneal cysts are disseminated in the abdominal cavity and become implanted at new sites.

(4) Expansions of hydatid cysts establish new sites in other visceral organs by contact.

Measurement: The choice of a parameter for an accurate measurement of the growth of larval E. multilocularis is difficult. Criteria such as the presence of protoscoleces and their numbers cannot be used because the development of protoscoleces depends on the species and the strain of the host rather than on the growth rate of the cysts. The volume and the weight of the cysts which

always contain some host tissues were nevertheless used to measure the growth of larval E. multilocularis (Lubinsky, 1960; Lubinsky and Dresser, 1963).

Location of cysts: In primary infections, cysts are mostly located in the liver. In animals injected intraperitoneally with cyst material, the hydatid cysts are mostly attached to the omenta and mesentaries; but the cysts often develop in the liver. Other common sites are the diaphragm, the parietal peritoneal and the intestinal wall. The cysts are seldom found in the spleen, the kidney and the lung (Mankau, 1955).

f. Factors affecting the growth in laboratory mice

Many factors affect the growth of larval E. multilocularis in laboratory mice.

Strain of the host: Yamashita (1958) pointed out the importance of strain differences in laboratory animals. Yamashita et al (1963) demonstrated that the 5 strains of mice studied, namely fm, NC, SM, gpc and KK, varied greatly in their susceptibility to oral infections. The growth of Echinococcus cysts in different strains and hybrids of Jackson Laboratory mice were studied by Lubinsky (1964 and 1966); Lubinsky and Desser (1963). The observation that in C57L and DBA_1 mice the growth of the cysts was fast, resulted in the production of LD_1F_1 hybrids in which the cyst growth is extremely rapid (Lubinsky, 1966; Fig. 3).

Figure 3

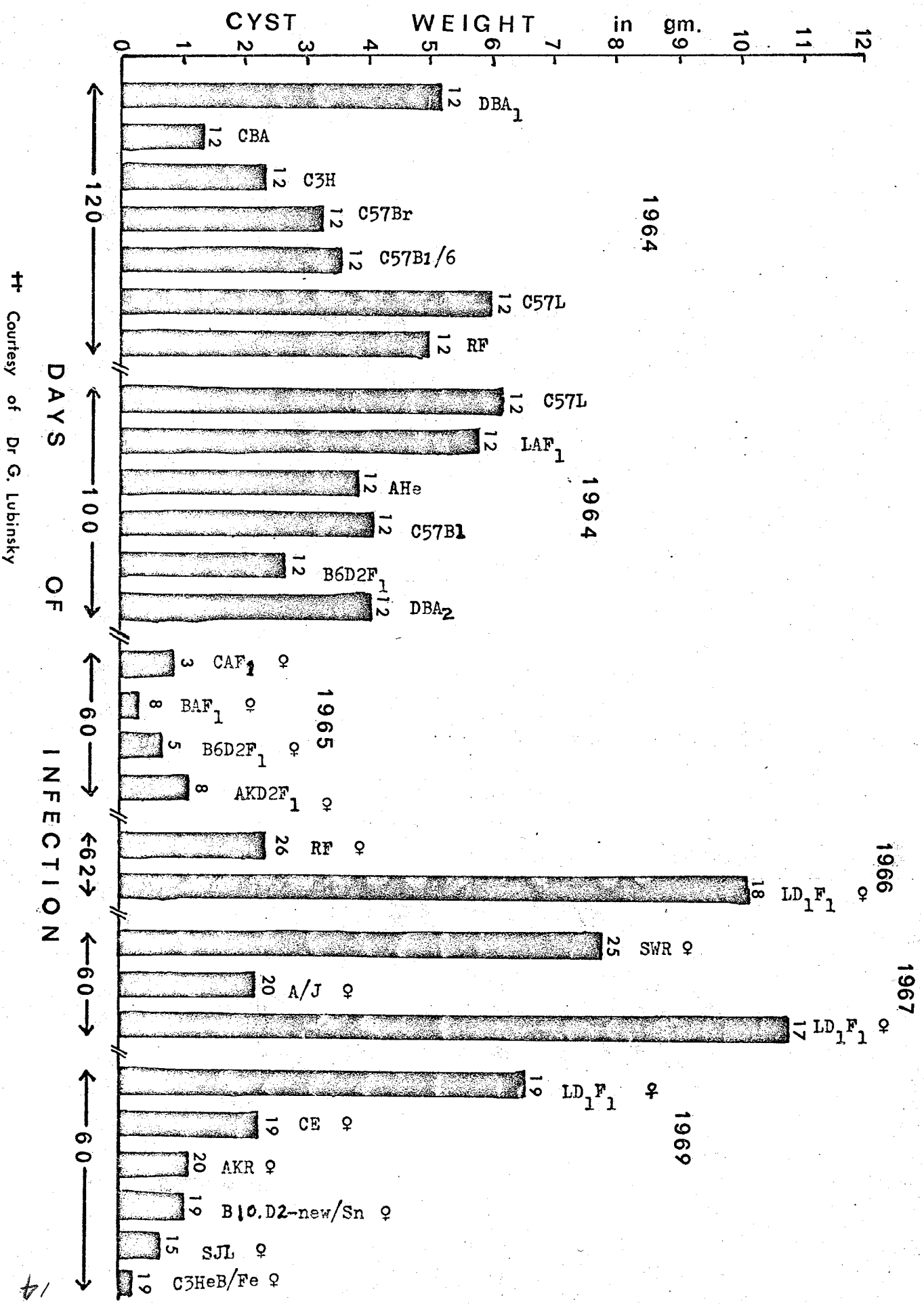
Effect of mouse strain on the growth of Larval Echinococcus multilocularis

The two parental strains of LD_1F_1 hybrids are female C57L and male DBA_1 mice. In LD_1F_1 mice, the intraperitoneal Echinococcus cysts grow extremely fast. These hybrids serve as an efficient biological tool in chemotherapy.

The number over each bar indicates the number of experimental animals used.

Courtesy of Dr. G. Lubinsky.

Figure 3



†† Courtesy of Dr G. Lubinsky

Host sex: Ohbayashi and Sakamoto (1966) demonstrated sex differences in susceptibility of KK and NC mice to infections with onchospheres of E. multilocularis, the females of both strains being more resistant than the males. However, Echinococcus cysts grow faster in the female A/J mice and LD_1F_1 hybrids than in males (Lubinsky; Lubinsky, 1966).

Host Age: No previous work on the effect of age on the growth of E. multilocularis has been published. Unpublished preliminary experiments of Lubinsky showed that the mean weight of Echinococcus cysts in 44 two-month old mice was 1.75 gm. whereas that in the 36 twenty-four month old mice was 2.90 gm. Schwabe et al (1959) showed that the resistance to secondary infection with E. granulosus increased with progressing age of white mice: mice 48 days of age or younger were highly susceptible while those 71 days or older were relatively resistant. The problem needs further study.

Host activity: Sigurdson (1969) demonstrated that the activity of infected LD_1F_1 mice as measured with an activity wheel reached a plateau four weeks after infection and later declined. I suggest that the decline in activity coincides with the second phase (acceleration) of the growth of the intraperitoneal Echinococcus cysts. The decline in activity is due to the progressive physical burden of the parasite.

Dose and nature of the inoculum: The larger the inoculum, the faster is the growth of the hydatid cysts. The larger the dose, the shorter is the incubation period, that is, the interval between

inoculation and the development of palpable cysts. The presence of protoscoleces in the inoculum is not essential for the transferability of E. multilocularis cysts to other animals. Fertile cysts developed in cotton rats within 20 days (a normal period) after intraperitoneal infection of acephalic cyst material from a gerbil (Lubinsky, 1969).

Route of administration: In most mouse strains and hybrids including LD_1F_1 and DBA_1, the intraperitoneal cysts grow faster than the subcutaneous cysts, A/J being an exception (Lubinsky, 1966). The statement that DBA and C57BL/6 mice were less susceptible to E. multilocularis infection by intraperitoneal route than by the oral route (Yamashita and Ohbayashi, 1960) is disputable.

2. The Reticuloendothelial System

a. History and Definition

The history of the reticuloendothelial system (RES) is given according to Reback et al (1960), Garnham (1963) and Florey et al, (1962). In 1891, Metchnikoff divided the cells of general defense system into hematogenous and histogenous macrophages which disposed of noxious agents. In 1904 Ribbert injected lithium carmine into the circulation of mammals and found that considerable amounts of it were taken up by certain cells. Aschoff (1924) coined the term "reticuloendothelial system" to include cells that absorb vital dyes. His definition included fibroblasts but not lymphocytes. The fibroblasts can take up dyes but do not have the capacity to concentrate them. Other authors like Tschaschin, Maximow and

Dougherty, demonstrated that lymphocytes in inflammatory lesions and tissue cultures are capable of ingesting and storing such dyes. In 1927 Maximow outlined the histological features of RES. In 1937, Taliaferro and Mulligan proposed the term "lymphoid-macrophage system" (LMS) to include not only macrophages but also macrophage precursors, namely lymphoid cells. Maximow and Bloom used the term "macrophage system" in their "Textbook of Histology" in 1952.

Obviously the term "reticuloendothelial system" is an abstraction as it regards the defensive system of the body as a unit.

The term RES is defined in this paper as a system of heterogenous cells, scattered throughout the body and mesodermal in origin, having various pathological, immunological and physiological functions, of which phagocytosis is the common characteristic.

b. Structure of the RES

Two items are described here: (1) the RES and (2) the omentum. The latter is added because of its importance in intra-peritoneal infection.

The RES is composed of two kinds of macrophages, the free and the fixed cells. Free macrophages include lymphocytes in tissue spaces, tissue histiocytes, lymphocytes, monocytes and other leukocytes in the blood, and also the pulmonary septal cells. Fixed macrophages are divided into two groups: the sinusoidal lining cells and the fixed macrophages in the tissues. The sinusoidal cells include Kupffer cells in the liver and similar cells in the spleen, corpus luteum, bone marrow, lymph nodes and the adrenal cortex. The fixed macrophages in tissues include certain cells of

lymph nodules, thymic stromal cells and microglia of the central nervous system.

A quantitative distribution of phagocytic cells in different organs is given by Snell (1960) as follows: Liver 60%, spleen 5% and other (lymph nodes, glands, circulation, etc.) 35%. The injected colloid, however, is not localized in a direct proportion to the number of phagocytic cells present. Liver is the major site. Together with the spleen, the liver removes generally 90% of the total injected colloidal carbon (Biozzi et al, 1953). The percentage distribution of cells in liver tissue of both mice and rats is as follows: parenchyma cells 60.6%, littoral cells or RE cells 33.4%, bile duct cells 2%, connective tissue cells 2.2%, and blood vessel wall cells 1.8% (Daost, 1958, after Berliner and Dougherty, 1960).

The omentum is an important part of the serous membranes of the peritoneal cavity. Each membrane is a thin layer of loose connective tissue covered by a layer of mesothelium. When the membranes are folded, serous cavities are formed and contain serous exudate. Holes are found in some areas of the omentum where they are only a network of non-vascularized collagenous bundles covered by mesothelial cells. In other areas of the omentum, undifferentiated cells occur along the blood vessels; histiocytes are abundant and small lymphocytes, plasma cells, eosinophils and mast cells are present. In certain areas, histiocytes accumulate in dense masses, macroscopically visible as round or oval patches, the so-called "taches laiteuses" which are especially

numerous in rabbits. It is mainly the action of the histiocytes of the omentum that makes it become jet black after an intraperitoneal injection of carbon suspension while the rest of the peritoneum is of normal color (Florey and Gowans, 1962). Free cells in the peritoneal exudate originate from the serous membranes. They are free macrophages, desquamated mesothelial cells (which may develop into fibroblasts in inflammation), small lymphocytes (from the migration through the blood vessels and proliferation centres of the omentum), eosinophils, tissue mast cells and, during inflammation, hematogenous heterophil leucocytes (Bloom & Fawcett, 1964). The omentum is thus basically a combination of macrophages and connective tissues.

c. Functions of the RES

It is extremely difficult to summarize the functions of the RES because of the diversity of cell types involved. The picture is further complicated by cell transformation.

The essential functions of the RES are described below under four headings.

Pathological activities: Phagocytosis is the common characteristic. The cells ingest particulate matter, such as micro-organisms, tissue debris and foreign injected colloidal particles dependent on opsonic factor. In many cases, they can inhibit the growth of ingested microorganisms and digest them. Another function of at least some of the RE cells is to act as progenitors of many highly differentiated cell types. For example, the lymphocytes of man are capable of being transformed into macrophages (Torre & Leiken, 1959). Some RE cells participate in inflammatory reactions. In the

acute phase of inflammation the cellular infiltrate consists of neutrophils and eosinophils. Granulomatous inflammation is a reaction of the RES to irritations including chronic infections. The cells involved are mainly of histogenous origin and the histiocyte is the characteristic cell. Neutrophils gradually disappear due to the increasing acidity of the inflamed area. Epithelioid cells are merely mature polyhedral transformed histiocytes (Lurie, 1960). They may fuse, forming large multinucleated giant cells. The accumulation of mononuclear histiocytes and their derivatives results in the formation of granulomata.

Immunological activities: Wissler et al (1960) asserted the participation of macrophages, lymphocytes and plasma cells in antibody formation. One of the recent theories is that of a two cell synergism in immune competence (Paraskevas, 1969). It is based on experiments with sheep RBC and bovine serum albumin (BSA) in mice. The antigen reactive cell (ARC) is a small lymphocyte, of long life span, which interacts with the antigen but produces no antibody; this ARC is present mainly in the blood and the periarteriolar sheaths in the spleen. The antibody producing cell (APC) is a small lymphocyte, of short life span, which differentiates into a mature plasma cell under the influence of ARC; it is present mainly in the lymphoid follicles of the spleen and the germinal centres of the lymph nodes.

Physiological activities: The RES may be intimately connected with iron metabolism (Florey, 1962). Kupffer cells and lymphocytes

are the two major types of RE cells involved in steroid metabolism (Berliner & Dougherty, 1960). Part of the cholesterol is preferentially taken up by Kupffer cells which may induce hyperlipemia and hypercholesterolemia by displacement of cholesterol to the blood. The lymphocyte has the ability to oxidize the 11 position of cortisol to form cortisone and to reduce cortisone to cortisol. Cortisol is lymphocytokaryorrhectic.

Role of the RES in chemotherapy: Goble and Singer (1960) mentioned that in 1924 Rosenthal and Spitzer and later investigators stressed the importance of an intact RES to the development of drug action in protozoan infections, especially in those whose course can be modified by the blockade of the RES and by splenectomy.

d. Experimentation on RES activities

Studies on RES activities may identify or correlate a particular RES activity with a certain effective defense mechanism of the host towards a specific agent.

Measurement of RES activities: The RES activities may result in qualitative and quantitative morphological changes (Taliaferro, 1956; Kojima, 1960; Machado et al, 1968). Quantitative studies may include: (1) in vivo blood clearance of colloids, such as carbon particles, chromium phosphate, thorium dioxide, lipopolysaccharides; and of larger particles such as bacteria and red blood cell stromata (Biozzi et al, 1953; Snell, 1960; Baillif, 1960; Freedman, 1960; Wright, 1927 after Carpenter 1965; Ingraham, 1955).

(2) Antibody production (Carpenter, 1965) and plaque formation for estimating antibody producing cells (Jerne & Mordin, 1963).

(3) Physiological activity of RE cells. Different types of RE cells can be isolated for such studies. Examples are Kupffer cells, lymphocytes, neutrophils, monocytes (Berliner & Dougherty, 1960; Ford, 1968; Morseth and Soulsby, 1969; Carpenter, 1965).

Depression of RES activities: Some common methods are as follows:

(1) Blockade of the RES. Particles of inert matter are injected into the body to saturate and impair the RE cells.

(2) Surgery. Removal of organs rich in RE cells, e.g. the spleen, implies the deprivation of the experimental animals of part of the structural basis of resistance. Neonatal thymectomy results in an impairment of the development and competence of the lymphoid system (Miller, 1961).

(3) Hormones. Cortisol and related 17-hydroxy-corticosteroids have antiphlogistic effects (Selye and Horava, 1953; Travis & Sayers, 1967). Thyroidectomy lowers and retards antibody formation but has no effect on blood clearance in rabbits injected with tubercle bacilli (Lurie, 1960).

(4) Chemicals. Simple organic compounds, such as palmitic acid may seriously affect the "lymphoid-macrophage system" (Garnham, 1963). The production of the (7S) fraction of antibody is inhibited by 6-mercaptopurine (6MP) (Calabresi & Welch, 1967). Iodoacetate inhibits the *in vitro* phagocytosis in rabbits by blocking glycolysis (Cohn & Morse, 1960). Snell (1960) reviewed the depressing effects of some antibiotics, vitamins and antihistamines on the RES.

Actinomycin-D inhibits antibody formation by blocking the RNA synthesis (Abramoff et al, 1968), but it has no effect on carbon clearance (Agarwal & Berry, 1968). Assessment of the effects of vitamins on the RES is difficult due to their general actions on nutrition.

(5) X-irradiation. Blaustein (1963) summarized the effects of total body irridiation on the blood cells in the rabbits. Within 9 days after 800r irridiation, general cessation of cytopoiesis of blood cells, intense destruction of lymphocytes and atrophy of lymphoid organs were evident. Active phagocytosis by littoral cells of the splenic sinuses was observed 24 to 48 hours after irridation. Cytopoietic activities were resumed within 9 to 14 days with exceptionally active lymphocytogenesis.

(6) Antireticular cytotoxic sera. In using such sera, Ekzemplarskaya (1945) was able to enhance parasitemia in experimental monkeys infected with Plasmodium inui, and also to produce relapses of latent infections.

(7) Intercurrent infections. Goble and Singer (1960) reviewed the effects of this method in malaria and trypanosomiasis.

(8) Physiological factors. The role of hypoxia (Goble and Singer, 1960), and diet (Newberne et al, 1968) were studied. Hypoxia produced relapses and interfered with the effectiveness of drugs in avian malaria.

Stimulation of the RES: Depending on certain factors, e.g. concentration, the same agent may act both as a depressor and a stimulant. Common stimulating agents are as follows:

(1) Hormones. Desoxycorticosterone acetate (DCA) enhances inflammatory reactions (Selye and Horava, 1953). Cortisone and cortisol at levels below 5 mg./kg. increase blood clearance of chromium sulphate in mice; the effect lasts for over 24 hours (Snell, 1960). Hyperthyroidism, induced by L-triiodothyroxine (TIT) and L-thyroxine produces reticular hyperplasia of lymphatic organs, enhances antibody formation but has no effect on blood clearance (Dougherty, 1952; Lurie, 1960). Among estrogens, diethylstilbestrol is the strongest phagocytic stimulant (Heller et al, 1957; Nicol et al, 1962).

(2) Chemicals. Chemical RES stimulants are numerous. Recent investigators tend to test simple chemicals of known structure (Machado et al, 1968). A single injection of restim, a simple lipid, produces a prompt hyperphagocytic state which lasts for several days; it does not cause granulomatous or plasma cell reactions (Heller, 1960). Dextran induces endothelial activation (Gözsy & Kato, 1960; Nicol et al, 1962).

(3) Biological derivatives of unknown structure. The more important are Bacilles-Calmette-Guerin, i.e. B.C.G. (Howard et al, 1959), antigenic macromolecules (lipopolysaccharides or heated denatured albumin globulin complexes) and cell wall extracts of yeasts, the "zymosan" (Benacerraf & Sebastyen, 1957 after Di Luzio, 1960; Machado et al, 1968). All enhance blood clearance of carbon. Yet both B.C.G. and zymosan cause the production of granulomata in the liver.

Care should be taken in interpreting the results of experiments with the RES. The agent used may act on systems other than the RES.

3. Indian ink blockade

A suspension of carbon particles is the commonest agent used to bring about a blockade of the reticuloendothelial system. This method was used since the 1920's to depress host resistance to various pathogens (Goble and Singer, 1960). The research was basically histological up to 1951 when the Halpern group in France developed techniques to study blood clearance and distribution of carbon particles in various organs (Halpern et al, 1953). The Indian ink blockade is also used in studies on antibody formation in microbial infections (Carpenter, 1965).

a. Effects on the host

Effects of RE blockade: The blockade causes the following effects: (1) RE exhaustion. Baillif (1960) reviewed the effects of repeated injections of colloidal thorium dioxide. At first there is a progressive mobilization of "phagocyticopotential cells" throughout the body which provides activated macrophages. Toxic changes may result in a progressive loss of appetite, weight and degrees of physical activity. This state can persist for a long time even if injections are discontinued. The animals show increased haemapoietic activity in the bone marrow and myeloid metaplasia in the spleen, liver and lymph nodes. The rodent spleen becomes largely granulopoietic. If the injections are continued the animals will die. In such cases, the post mortem findings are:
(a) a decrease in cellularity of the marrow and a depletion of lymphocytopoietic organs of lymphocytes: (b) the presence of free clumps of thorium dioxide granules in various organs; and

(c) the presence of only a few newly activated macrophages that contain small numbers of phagocytosed granules.

(2) Inhibition of phagocytosis. Banacerraf and Miescher (1960) reviewed the kinetics of in vivo blood clearance of carbon. In rats, the RES is saturated with a standard dose of 16 mg. of carbon per 100 gms of body weight. At such a blockading dose, the commercial Pelikan Indian Ink depressed the phagocytic activity for 12 days. The specially prepared carbon suspension (C11/1431a, Guenther Wagner) from the same manufacturer depressed the phagocytic activity for only 3 days (Benacerraf et al, 1954).

(3) Inhibition of antibody formation. Animals blocked with Indian ink and subsequently injected with an antigen often produce less antibody than the controls (Carpenter, 1965; Wissler et al, 1960).

(4) Vulnerability and adaptative potential of the RE cells. RE cells with a great amount of phagocytosed particles are more fragile (Rindani et al, 1953). These authors suggested that Indian ink treatment orients macrophages into the direction of phagocytes and thus limits their potential of cell transformation. Inflammatory reactions did not seem to be affected.

The extent and character of host responses to the RES blockade depend on the species of the host, additional ingredients in the suspension such as phenol and shellac (Halpern et al, 1953), regenerative capacity of the RES (Benacerraf et al, 1954), the dose and the size of particles (Biozzi et al, 1953).

Fate of injected carbon: Within 4 hours after intraperitoneal injection of Indian ink only a few aggregates of carbon particles

were found on the surface of the liver and spleen; most of them were concentrated on the omenta, mesenteries and in the retroperitoneal tissues (Spain et al, 1950). Omental lymph nodes are the primary foci of Indian ink concentrations (Florey and Gowans, 1962). Most of the particles are at first extracellular, but become phagocytosed later on (Magee and Palmer, 1953). Halpern et al (1953) studied the fate of carbon particles administered intravenously. In the rats which received 16 mg. carbon/100 gm. of body weight, almost all the carbon disappeared from the blood stream within one hour. Ninety percent of it was found in the liver and the spleen. In rats which received higher doses, the amount of carbon recovered from the organs decreased. A substantial portion of carbon was found in the lungs, but later moved to the spleen and the liver. Benacerraf et al (1954) further showed histologically that the carbon, phagocytosed by Kupffer cells and at first evenly distributed in the liver, later migrated to the periportal spaces, leaving the cells of the lobules free from it. This shift is completed within one month after the injection of the carbon suspension.

b. Parasitic infections

Indian ink blockade lowers the functions of the RES, especially the phagocytosis. Garnham (1963) discussed the influence of the blockade of the RES with Indian ink and other substances on protozoan infections. The blockade does suppress immunity to some avian plasmodia and does provoke relapses, but the results vary greatly, especially in mammals.

Goble and Singer (1960) reviewed the effect of the blockade of the RES on plasmodial and trypanosomal infections. The first studies on the influence of the blockade of the RES with Indian ink on protozoan infections were reported in 1927. Working with Trypanosoma brucei, Kritchewski (1927) and later investigators found that an intact RES was necessary for optimal drug action on the parasite. Browning et al (1934) tried to enhance T. congolense infections in mice using saccharated iron. They found that three out of six mice with blocked RES did not become infected at all. Taliaferro & Pavlinova (1936) worked with T. duttoni, which is nonpathogenic to mice. They found that both splenectomy and Indian ink blockade enhanced the parasitemia and prolonged the period of trypanosomal multiplication. The effects of the blockade on T. cruzi in rats and mice were inconclusive. However, Goble and Boyd (1957) were able to enhance T. cruzi infections in mice using thorium dioxide blockade. The treatment shortened the survival time of mice. Equivocal results were obtained with T. gambiense in mice (after Goble and Singer, 1960)

Schuurman et al (1929) obtained negative results with avian Plasmodium relictum and Indian ink blockade. Trager (1941) produced higher initial parasitemias with P. lophurae in chicks injected intraperitoneally with Indian ink. Thompson et al (1948) found that radio-active colloidal iron did not affect infections with P. cathemerium in canaries and P. lophurae in ducklings. Goble and Singer (1960) blocked CF1 mice with Indian ink; the results were a higher parasitemias of P. berghei infection and shorter survival time of these mice. Other colloids tested such as thorium dioxide, saccharated iron oxide and polyvinyl pyrrolidone did not exert such actions.

It is obvious that the blockade of the RES in most cases affects the course of infections with both trypanosomes and malarial plasmodia.

4. Splenectomy

a. The spleen

Structure: The spleen is the largest single lymphoreticular organ inserted in the blood stream. The course of the blood flow through the spleen is illustrated in Fig. 4B. The circulation is open in mice but is closed in rats and man (after Hummel et al, 1966).

Functions: Normal functions of the spleen are given in Fig. 4A. The spleen also acts as a blood filter. It cleans approximately 4% of the circulating blood volume per minute (Ellis and Smith, 1966).

The spleen possesses a combination of phagocytic, cytopoetic and antibody-forming capacities essential in immunity (Taliaferro, 1956).

It has a greater phagocytic activity per gm. of tissue than the liver (Ingraham, 1955). It is also the principal site of sequestration of sensitized RBC in the presence of low levels of opsonizing antibodies (Jandl and Kaplan, 1960).

The spleen is a lymph gland in which lymphocytes are produced. It is also an important centre of erythropoiesis in all mammalian embryos and some adult mammals, e.g. mice and rats.

Figure 4

The spleen

A. Normal functions of the spleen
(after Blaustein, 1963)
B. Histology of the spleen
(after Ham, 1963)

Figure 4A

THE SPLEEN

Cellular production	Function	Related disease
Lymphoblasts, lymphocytes	Phagocytosis Production of antibodies Labile protein and nucleic acid deposits Source of other cells (plasma cells, fibroblasts)	Boeck's sarcoid Infectious lymphocytosis Infectious mononucleosis Acute and chronic lymphatic leukemia
Reticuloendothelial cells:		
Plasma cells	Source of immune globulin Participates in endogenous protein metabolism Phagocytosis	Multiple myeloma Plasma cell leukemia
Monocytes	Phagocytosis Role in water and fat metabolism	Infectious mononucleosis Monocytic leukemia
Other	Culling effect on red blood cells Role in iron metabolism (sinusoidal epithelium) Pitting function on red blood cells Role in phagocytosis of senile and worn out red blood cells Cellular debris Platelets Nuclear and cytoplasmic remnants of leukocytes	Lipid storage diseases: Gaucher's Niemann-Pick Letterer-Siwe Diabetic lipemia Reticuloendotheliosis Reticulum cell sarcoma

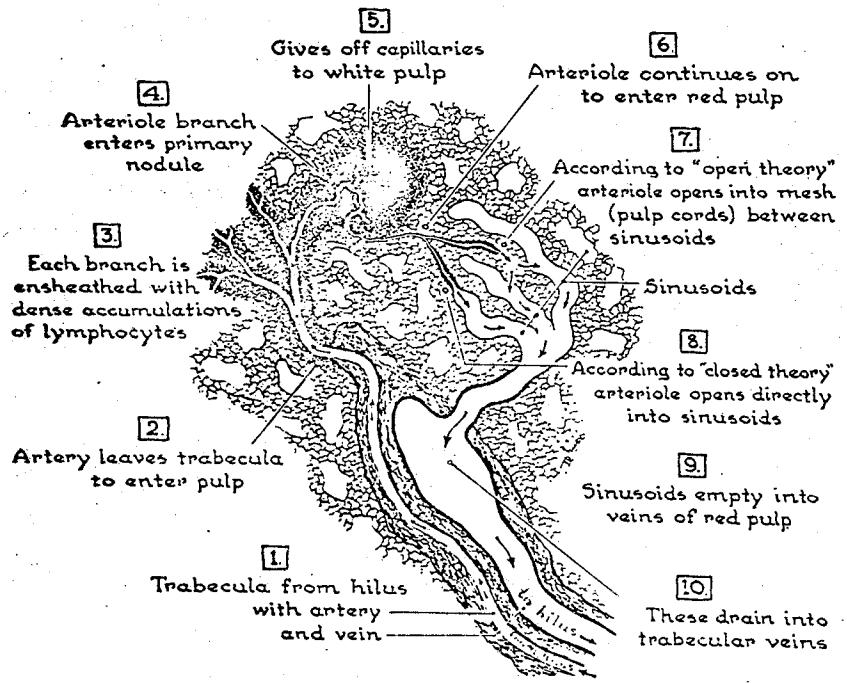


FIG. 4B Diagram of the course of blood through the spleen according to the open and closed theories of circulation. The legends on the figure should be read in a clockwise fashion.

Figure 4

Mesenchymal cellular reserves in the postnatal animals consist of predominantely fixed reticular cells and lymphocytes, and, in many species, various lymphoid stem cells of the myeloid series.

Already in 1899, Deutsch recognized the spleen as the major reservoir of antigenic material capable of stimulating prolonged antibody formation (after Carpenter, 1965). Taliaferro (1956) found that the spleen is initially the most active organ per unit weight in the formation of antibodies against antigens reaching the blood, but rapidly falls off in antibody production.

Splenomegaly An enlargement of the spleen can be induced by parasitic infections, such as malaria, leishmaniasis, schistosomiasis and echinococcosis. A 5-fold increase in weight is not uncommon. The condition is the result of an interplay of depletion of macrophages and of hyperplasia (Taliaferro, 1956).

b. Effects of splenectomy on the host

Physiologically the spleen is disposable. Splenectomy is even recommended in some internal and hereditary diseases (Slattery, 1963). Splenic functions, such as iron and fat metabolism and erythrolysis are taken over by other organs, namely the liver, bone marrow and the lymph nodes (Bloom and Fawcet, 1964).

Splenectomy results in a loss of the major erythrocyte-clearing organ. It temporarily lowers the macrophage activity. After splenectomy total leucocyte count in the blood increases and a distinct lymphocytosis, neutrophilia and eosinophilia develop (Bloom and Fawcett, 1964; Ford, 1968). But these phenomena soon

disappear. After an intravenous injection of particulate antigen, only low titres of antibody appear in splenectomized rats, man or the rabbit (Rowley, 1950; Draper and Süssdorf, 1957). Splenectomized rabbits respond to continual antigenic stimulation with a delayed and yet higher antibody titre than the controls (Taliaferro, 1956).

Most authors assert that splenectomy causes an immediate depression of both innate and acquired immunity. Since the first report on the high incidence of serious bacterial infection in splenectomized infants was published by King and Schumaker in 1952, a considerable literature has accumulated. Ellis & Smith (1966) reviewed this problem and pointed out the importance of certain humoral antibodies produced by the infantile spleen, which facilitates the phagocytosis of pneumococci and other microorganisms.

c. Effects on parasitic infections

Introduction: According to Nooruddin & Ahmed (1967) the first experimental splenectomy was carried out by Zambeccari in 1680 on a dog. The importance of splenic macrophages in malaria was studied as early as 1884 (after Taliaferro, 1956). Plimmer and Bradford (1899) were the first to study Trypanosoma brucei infections in splenectomized dogs, cats and rabbits. The study of the role of splenectomy in simian malaria by von Berenberg-Gossler (1909) antedated its use in avian malaria by 30 years (after Goble and Singer, 1960).

Three striking results of splenectomy are (1) activation of latent infections, (2) enhancement of the susceptibility of refractory or resistant hosts to infection and (3) enhancement of

athreptic immunity by interfering with erythropoiesis and thus limiting the percentage of young erythrocytes which are the most desirable substrate for many protozoans, especially Plasmodium berghei (review by Goble and Singer, 1960). The effect may be greatly enhanced if splenectomy is combined with "blockade" with particulate matter (Taliaferro et al, 1931; Taliaferro and Pavlinova, 1936).

The depression of innate and acquired immunity by splenectomy is not a general rule. It seems to be limited to diseases which severly affect the macrophage tissue of the spleen. Nooruddin and Ahmed (1967) reviewed many factors which may cause such inconsistency; host species, its age and sex (Zuckermann and Yoeli, (1954), the site, route and quantity of the antigen injected, the interval between splenectomy and infection, the species of the parasite, and its life stages.

In general, the spleen has a greater protective value in the rat, mouse, monkey and dog than in the rabbit or guinea pig. In susceptible splenectomized hosts rickettsial and protozoan infections run a more acute course than those caused by metazoan parasites.

Subprotozoan infections: In calves infected with Anaplasma marginale splenectomy causes a fatal parasitemia, delaying the production of macroglobulin and complement fixing antibody, and depressed the production of immune globulin (Ig G) (Klaus and Jones, 1968). Splenectomy in rats is often followed by recrudescence of chronic Bartonella muris infections (Dingle, 1941; Falkner and Haberman, 1957; Bloom and Fawcett, 1964).

The piroplasms, the erythrocytic parasite of mammals, almost never infects man. However, in the three human cases reported, all the patients had been previously splenectomized before they died of infection (Škrabalo and Deanovic, 1957; Fitzpatrick et al, 1968; Scholtens et al, 1968;). Todorovic et al (1967) reviewed the effects of splenectomy on the course of babesiasis (Table I).

Malarial infection:- The earliest experiments on the influence of splenectomy work on the course of malaria was done on monkeys (Gonder and Bondenwalt, 1910; Leger et al, 1912; Blanchard and Langeron, 1913 and Bouilliez, 1913). Taliaferro (1948) and Taliaferro & Kelsey (1948) studied the role of the spleen and lymphoid macrophage system in quinine treatment of Plasmodium gallinaceum infections in chicks. They concluded that 3 anti-malarial factors operate independently in this system, namely innate immunity, acquired immunity and quinine. El-Nahal (1966) observed a non-fatal recrudescence in the blood of splenectomized chickens infected with P. gallinaceum. Antibody titre rose after splenectomy to a higher level than the controls. Elks and Cantrell (1966) observed the effects of splenectomy on the phagocytosis of i.v. injected colloidal carbon in rats infected with P. berghei. The effect of pre-infection splenectomy on the phagocytosis was negligible in the course of the first seven days after infection. Later the phagocytosis in the splenectomized animals was much lower than in the controls. Elk and Cantrell suggested that the spleen is not necessary for the development of immunity but is important for the maintenance of enhanced RE activity in malarial infection.

TABLE I

Effect of Splenectomy on the Course of Plasmodial and Babesial Infections of Mammals and Birds

Host	Parasite	Results after splenectomy	Author(s)
Man	*Babesia bovis*	Parasitemia and death	Skrabalo and Deanovic (1957)
	Babesia species	Parasitemia	Braff and Condit (1967)
Chimpanzee	*Plasmodium vivax*	Parasitemia	Bray (1957, 1958)
	P. falciparum	Parasitemia	Bray (1957, 1958)
	P. ovale	Parasitemia	Bray (1957, 1958)
	B. divergens	Parasitemia, hemoglobinuria	Garnham and Bray (1959)
	B. bovis	Parasitemia, hemoglobinuria	Garnham and Bray (1959)
Monkey	*P. kochi*	Relapse	Gonder and Rodenwalt (1910)
	P. inui	Parasitemia and death	Krishnan *et al.* (1933)
	B. divergens	Parasitemia and death	Garnham and Bray (1959)
	B. bovis	Parasitemia and death	Garnham and Bray (1959)
Rabbit	*B. divergens*	Insusceptible	Garnham and Bray (1959)
	B. bovis	Insusceptible	Garnham and Bray (1959)
Rat	*P. berghei*	Parasitemia and death	Fabiani and Fulchiron (1954)
		Parasitemia and death	Galliard (1949)
		Parasitemia and death	Galliard and Lapierre (1950)
		Parasitemia and death	Zuckerman and Yoeli (1954)
		Parasitemia and death	Prakash (1961)
		Parasitemia and death	Cantrell and Moss (1963)
		Parasitemia and death	Spira and Zuckerman (1965)
Mouse	*P. berghei*	Parasitemia and death	Singer (1954)
		Parasitemia and death	Kretschmar and Jerusalem (1963)
Hamster	*P. berghei*	Low parasitemia, more susceptible	Carrescia (1961)
	P. vinckei	Higher parasitemia, low mortality	Adler and Foner (1961)
Ground squirrel	*B. graingeri*	Parasitemia (new species)	Heisch (1952)
Genet cat	*B. genetae*	Parasitemia (new species)	Heisch (1952)
Buffalo	*B. bigemina*	Parasitemia and death	Miessner (1931)
		Parasitemia and death	Galliard and Cebe (1941)
Cattle	*B. argentina*	Parasitemia and death	Galliard and Cebe (1941)
Canary	*P. rouxi*	Lower parasitemia, more resistant	Corradetti and Verolini (1958)
	P. cathemerium	No influence	Causey (1939)
Duck	*P. elongatum*	No influence	Manwell *et al.* (1957)
	P. circumflexum	No influence	Manwell *et al.* (1957)
Chicken	*P. gallinaceum*	No influence, prior to infection; higher parasitemia with latent infection	Terzian (1946)
	P. gallinaceum	Lowered parasitemia	Taliaferro and Kelsey (1948)
	P. gallinaceum	Parasitemia, recrudescence	El Nahal (1966)
	P. juxtanucleare	Recrudescence, mortality	Al-Dabagh (1960)

Reprinted from an article by Todorovic et al, 1967. Exp. Parasit. 21: 354-372.

Effects of splenectomy on Plasmodium infections in mammals and birds are summarized as follows (after Goble and Singer, 1960 and Todorovic et al, 1967):

Primates	chimpanzee	Plasmodium vivax	Higher parasitemia
		P. falciparum	Higher parasitemia
		P. ovale	Parasitemia
	monkey	P. kochi	Relapse
		P. inui	Parasitemia and death
		P. cynomolgi	Higher parasitemia and relapse
Birds	canary	P. cathemerium	No effect
		P. rouxi	Lower parasitemia; resistant
		P. relictum	No effect
	duck	P. cathemerium	No effect
		P. circumflexum	No effect
	chicken	P. gallinaceum	Pre-infection splenectomy - no effect; relapse in latent infection.
		P. circumflexum	transient infection
		P. juxtanucleare	recrudescence and death
Rodents	rat	P. berghei	Parasitemia and death
		P. gallinaceum	Parasitemia and death
	mouse	P. berghei	Higher parasitemia and death
	Microtus guentheri	P. berghei	Higher parasitemia and death
	cotton rat	P. berghei	Recrudescence
	hamster	P. berghei	Low parasitemia and more susceptible
		P. vinckei	Higher parasitemia and lower mortality

Trypanosomal infection The influence of splenectomy on trypanosomiasis was studied as early as 1899 by Plimmer and Bradford (after Goble and Singer, 1960). The results were more contradictory. Effects of experimental splenectomy on trypanosomiasis are summarized as follows (after Goble and Singer, 1960):

Carnivores	dog	Trypanosoma brucei	Equivocal
		T. gambiense	Higher parasitemia
		T. evansi	Parasitemia
		T. cruzi	No effect
	cat	T. brucei	Equivocal
		T. rhodesiense	No effect
Rodents	rat	T. brucei	Equivocal
		T. gambiense	Equivocal
		T. evansi	Equivocal
		T. lewisi	Longer parasitemia and death (splenectomy plus ink blockade)
		T. cruzi	No effect
		T. congolenses	Higher parasitemia
		T. simiae	No effect
		T. vivax	No effect
	mouse	T. gambiense	Little or no effect
		T. evansi	Equivocal
		T. equiperdum	No effect
		T. duttoni	Higher parasitemia and death (splenectomy plus ink blockade)
		T. cruzi	No effect
		T. congolense	Equivocal
		T. simiae	No effect
		T. vivax	Transient infection
	rabbit	T. brucei	Equivocal
		T. evansi	No effect
		T. simiae	Parasitemia and death
	guinea pig	T. brucei	Parasitemia
		T. evansi	No effect
		T. equiperdum	No effect
		T. cruzi	No effect
		T. simiae	No effect
	harvest mouse	T. lewisi	Transient infection

Helminthic infections Only a few experiments on the effects of splenectomy on helminthic infections have been published. Except for filariasis, the effect of splenectomy on both innate and acquired immunity to helminths is not obvious. However, the spleen does play a certain role in the development of immunity to metazoan infections. A direct proof was given by Friedberg et al (1968) who used the spleen cell transfer technique in mice infected with Hymenolepis nana. The earliest experiments on the influence of splenectomy on helminthic

infections were those of Bittner (1913) who experimented with trichinosis in rabbits.

Thompson (1954) found that albino mice and golden hamsters were susceptible to Schistosoma mansoni infections while albino rats and guinea pigs were resistant. Splenectomy in mice and rats did not affect the course of initial infections or reinfections. Bruce et al (1966) found that antibody titres were not associated with severity of infection and acquisition of immunity in rhesus monkeys infected with S. mansoni. The serological and pathological aspects of schistosomiasis were not obviously altered by splenectomy either before or after exposure to infection with cercariae except that the splenectomized monkeys passed more eggs for a longer period of time.

Hoeppli (1941) stated that splenectomy enhanced slightly the susceptibility of a resistant strain of mice to Hydatigera taeniaeformis but the data is not clear cut. Larsh (1944) found no obvious effect of splenectomy on innate resistance of mice to Hymenolepis nana. According to Weinmann (1968), the involvement of antibodies in the immunity of mice to H. nana infections is proved by passive transfer experiments. This author found that there was essentially no difference between splenectomized, sham-operated, and untreated mice in the development of acquired immunity 3 and 6 days after an infection with H. nana eggs.

Mathies (1962) found that splenectomy does not alter the resistance of mice to Aspicularis tetraptera. Splenectomy did not affect the development of resistance of young rats to Nippostrongylus brasiliensis infections as measured by egg production, worm counts or antibody production. The results were essentially the same when

splenectomy was performed before initial infection, at the height of the initial infection or before reinfection (Ogilvie and Jones, 1967) Nooruddin and Ahmed (1967) reviewed the role of the spleen in filariasis. According to them, Duke in 1960 found that splenectomy caused a recrudescence of Loa loa infections in Mandrillus leucophaeus, expressed in high microfilarial counts which lasted for 12 months compared to 8 to 12 weeks in the controls. Hawking (1962) found that splenectomy caused little or no recrudescence in dogs infected with either Dirofilaria immitis or D. repens, and in gerbils infected with Dipetalonema witei. In cotton rats infected with Litomosoides carinii, splenectomy was followed by a minor increase in the microfilaria count in the blood. Hawking suggested that Dirofilaria spp. are well adapted to the dogs while L. loa is not well adapted to the monkey. In a search for a suitable laboratory host for Brugia malayi and B. pahangi, Ahmed in 1965 found that splenectomy did not cause parasitemia in guinea pigs and white mice (after Nooruddin & Ahmed, 1967). However, it was possible to establish B. malayi in 2 out of the 12 splenectomized Liverpool white rats but in none out of the 23 intact rats; and to establish B. pahangi in all four splenectomized Delhi white rats but only in 2 out of 6 intact rats.

Obviously, splenectomy in mammals does not markedly change the course of helminthic infections except that of Loa loa, a filariid. Results of experiments with the following helminths were reported:

TREMATODA	Digenea	Schistosoma mansoni	Thompson (1954) Bruce et al (1966)
CESTODA	Cyclophyllidea	Taenia taeniaeformis Hymenolepis nana	Hoeppli (1941) Larsh (1944) Weinmann (1968)

NEMATODA	Trichuroidea	Trichinella spiralis	Bittner (1913)
	Strongyloidea	Nippostonylus brasiliensis	Ogilvie & Jones (1967)
	Oxyuroidea	Aspicularis tetraptera	Mathies (1962)
	Filarioidea	Dirofilaria immitis	Hawking (1962)
		Dipetalonema witei	Hawking (1962)
		Loa loa	Duke (1960), after Nooruddin and Ahmed (1967)
		Litomosoides carinii	Hawking (1962)
		Brugia malayi	Ahmed (1965) after Nooruddin and Ahmed (1967)
		B. pahangi	Ahmed (1965) after Nooruddin and Ahmed (1967)

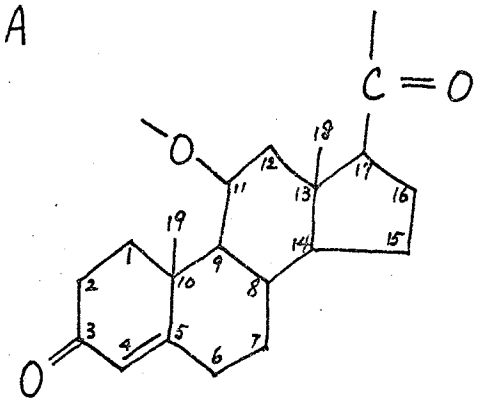
It is well known that antibodies are not efficient in controlling helminthiasis. The fact that helminths contain and produce many different antigens further complicates the immunological research on these parasites.

Any contribution to the study of this problem seems to be of both practical and academic interest. Splenectomy which delays the production of antibodies in mammals is a good method biological research.

5. Cortisone Acetate Treatment

a. Cortisone

Cortisone acetate (Cortone, Merck, Sharp & Dohme, Canada) is a synthetic 17-ketosteroid with the basic effects of cortisone. The zona fasciculata of the adrenal cortex produces largely cortisol from cholesterol. Cortisol (syn. hydrocortisone and Coumpound F) and cortisone are interconvertible by cellular metabolism, particularly in lymphocytes (Berliner & Dougherty, 1960; Fig. 5). Cortisol is the active antiphlogistic form. Its secretion is regulated by ACTH, and at least in part, by another hormone, angiotensin II (Travis & Sayers, 1967).


A
C=O
O
18
12
11
13
17
16
19
14
15
1
9
2
10
8
3
5
4
6
7
O

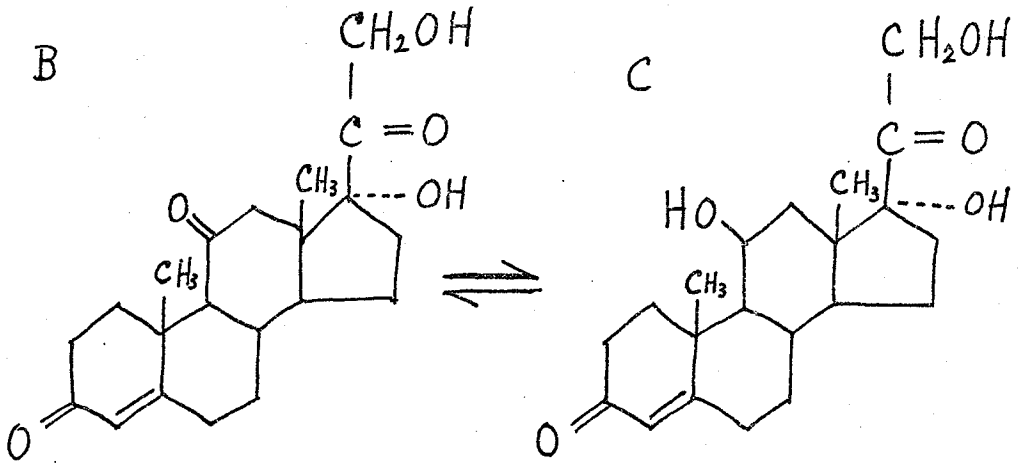

B
CH2OH
C=O
CH3
OH
O
CH3
O
C
CH2OH
C=O
CH3
OH
HO
CH3
O

Figure 5

In plasma, cortisol exists in a free form and a protein bound form. Cortisol has a higher affinity to plasma alpha globulin than to albumin. Biotransformation of the hormone takes place mainly in the liver. Extrahepatic metabolism, however, also occurs in fibroblasts, lymphocytes and even in the kidney. Metabolites are excreted in urine as compounds conjugated with glucuronic and sulphuric acids.

Basically cortisone and its 17-hydroxy analogs are glucocorticoids. Cortisone, cortisol and prednisole have a low sodium retaining activity. Methyl-prednisolone, triamcinolone, paramethasone, betamethasone and dexamethasone are devoid of such activity. They are all antiphlogistic (Travis & Sayers, 1967).

Cortisone was first introduced by Hench and Kendall in 1949 for the treatment of rheumatoid arthritis and inflammatory eye diseases. Corticosteroid therapy has also been recommended for treatment of certain collagen diseases, allergies, acquired hemolytic anemias and Addison's disease. It is also used now in immunosuppression.

b. Effects on the host

Physiological effects: Cortisone seems to decrease general physiological activities (Lurie, 1960). Some of its important effects are (1) an increase in glyconeogenesis, glycogen storage in the liver and in the excretion of nitrogen; (2) sodium retention by kidney, excretion of potassium and metabolic alkalosis; (3) accumulation of extracellular fluid; (4) decrease of capillary permeability (Menkin, 1953).

Pathological effects: Cortisone seems to act primarily on mesenchymal cells. Prolonged treatment with cortisone may depress the resistance

of the host and cause a systemic infection, either chronic or acute. Some important features are (1) phagocytosis. Snell (1960) showed that in C57Bl mice, a dose of 25 mg./kg. body weight produced marked inhibition of phagocytosis of chromium phosphate in the blood immediately after and one hour following the administration of this hormone, but the rate of phagocytosis returned to normal in 24 hours. A blocking dose may vary by 1 or 2 orders of magnitude depending on the strain of the mice. This may explain some contradictory reports published: no effect (Magnee and Palmer, 1953; Benacerraf, et al, 1954); inhibitory effects on phagocytosis both in the blood and the peritoneal cavity (Marmont and Biassoni, 1962; Florey, 1962).

(2) Anti-inflammation. Cortisone inhibits the earliest phenomena of inflammation (fibrin deposition, capillary dilation, migration of phagocytes into the inflamed area) and later manifestations (increased capillary permeability, fibroblast proliferation, deposition of collagen and cicatrization). Cortisone has no effect on the regeneration of mucin by epithelial cells after the induction of acute inflamation in the gastro-intestinal tract. Mast cells lose their granules and degenerate. The formation of granulation tissue is retarded by cortisone in the mouse, the rabbit and man, but not in the guinea pigs (Florey, 1962). The theory of Eyring and Dougherty on the mechanism of antiphlogistic effect of cortisone emphasizes the role of the fibroblasts (Berliner and Dougherty, 1960).

(3) Cellular changes in the blood. Cortisone causes lymphopenia, eosinopenia, a mild neutrophilic granulocytosis and increases the production of erythrocytes (Travis and Sayers, 1967).

(4) Lymphocytokaryorrhaxis. Cortisone inhibits lymphocytopoiesis and promotes cell destruction. These effects are not associated with

the extra-hepatic metabolism of the hormone. In the processes of lymphocyte destruction, the lymphocytes lose their cytoplasm, and the nuclei become pyknotic and eventually disintegrate. The mature lymphocytes are more susceptible than the immature (Dougherty et al, 1960). This effect of cortisone may account for the atrophy of lymphoid organs.

Immunological effects

Cortisone does not inhibit antibody production to any great extent in man, monkey or guinea pigs, but does so in rabbits, rats and mice (Florey, 1962). Raffel in 1961 stated that cortisone has no effect on the level of antibody already formed (Travis and Sayers, 1967).

c. Effects on parasitic infections

The basic effects of cortisone and of its analogs on parasitic infections are (1) enhancement of the susceptibility of resistant hosts to infection; (2) the suppression of acquired resistance of the host.

Contradictory results of experiments concerning the depression of the RES by cortisone in parasitic infections were sometimes reported. The discrepancies in the results obtained depend partially on factors such as species, age and sex of the host, site and route of infection, parasite species, and peculiarities of its life cycle, and the dosage of cortisone.

Protozoan infections Cortisone did not increase the susceptibility of weanling or older rats to infection with Entamoeba histolytica (Villarejos, 1962).

Jeffries and Harris (1967) studied the method of enhancement of trichomoniasis for chemotherapeutic studies. The severity of subcutaneous lesions produces by 6 freshly isolated strains of Trichomonas vaginalis and 2 out of 3 stock culture strains of T. foetus was enhanced by cortisone treatment.

According to Goble and Singer (1960), Wolf et al in 1951 found that cortisone activated latent infections with Trypanosoma cruzi in monkeys. The effects of cortisone on trypanosomal infections are summarized as follows (after Goble and Singer, 1960; Sherman and Puble, 1967):

		Trypanosoma cruzi	Activation of latent infection
Primates	monkey		
Rodents	mice	T. evansi	No effect
	rats	T. cruzi	Intense parasitemia and death
		T. rhodesiense	Parasitemia or resistance (dose and time dependent)
		T. lewisi	Intense parasitemia. No relapse

According to Goble and Singer (1960), Schmidt and Squire in 1951 were the first to study the effect of cortisone on malaria. The effects of cortisone on experimental plasmodial infections are summarized as follows (after Goble and Singer, 1960; Jackson, 1955; Roberts, 1954):

Primates	monkey	P. cynomolgi	Intensified parasitemia and relapse
Birds	pigeon	P. relictum	Longer parasitemia and death
	dove	P. gallinaceum	No effect
	chicken	P. gallinaceum	No effect
Rodents	rat	P. berghei *	Athreptic immunity in young rats.
	hamster	P. berghei	No effect
	mouse	P. berghei	No effect. * Athreptic immunity in young mice.

Athreptic immunity is an immunity based on the scarcity of

nutrients necessary for the development of a certain parasite. Cortisone, in high doses, causes a paucity of reticulocytes that are substrate of choice for some species of plasmodia. The shortage of young red cells results in a lower and slower increase of the number of plasmodia in mice. High doses of cortisone interfere with erythropoiesis as well as the RES activity.

Helminthic infections Cortisone appears to affect primarily certain stages in the life cycle of the helminthic parasite: (1) those which evoke strong tissue reactions, e.g. Trichinella (see Ritterson, 1959) and (2) those which exert strong antigenic stimulation, e.g. hexacanths of cestodes (See Silverman, 1965). However, cortisone does not always enhance helminthic infections.

Weinmann and Hunter (1960) studied the effects of cortisone on Schistosoma mansoni infection in mice. "There was a small but statistically significant reduction in worm burden in the majority of cortisone-treated mice. Essentially the same effect upon worm burden was produced whether the hormone was administered upto shortly before exposure to cercariae or commencing on the 15th day after infection when the parasites are in the liver or portal system". The above authors postulated a direct effect of cortisone on the early stages of development of the parasite.

Cortisone increased the susceptibility of rats to Paragonimus miyazakii infections but not to the more pathogenic P. westermani (Tada, 1967).

Weinmann and Rothman (1961) found that cortisone in doses as low as 0.05 mg. per mouse per day for one week impaired their

acquired immunity to Hymenolepis nana infections. This treatment allowed massive re-infection to occur when the primary adult worms were still present in the gut. The mice recovered the ability to resist re-infection within 4 to 6 days after the cortisone treatment was discontinued.

Oliver (1962) used cortisone to make resistant mice highly susceptible to Hydatigera taeniaeformis infections. He found that cortisone was effective only when given within the first 12 days of infection. He correlated this phenomenon to the production of antibodies by mice within the same period. Similar results were obtained by Esch (1967). Cortisone was found to have the greatest effect on the number of Taenia multiceps larvae when injected between the first and the 14th day after infection. He also found that cortisone increased the number of larvae in the subcutaneous tissue but not in the central nervous system.

Much more work has been done on the effect of cortisone on nematodes especially on Litomosoides and Trichinella.

hite rat is not a normal host to Litomosoides carinii. Cortisone suppressed the innate resistance of the white rat to this nematode and also the antibody production in this host (Briggs, 1963), but it did not increase the percentage of larvae completing migration to the lungs (Olson, 1959).

Campbell (1963) found a strain of albino mice which developed innate resistance to Trichuris muris by expulsion of immature worms 2 to 5 weeks after infection. The expulsion could be suppressed by a single dose of cortisone, either during the infection or 1 day prior to it.

Coker (1955a) studied the effects of cortisone on Trichinella spiralis in mice which developed a delayed acquired immunity to an initial infection with Trichinella spiralis. The immunity developed about 30 days after infection (Coker, 1956). Cortisone abolished this (Coker, 1955b). Zaiman et al (1962) studied the synergistic effect of heavy doses of T. spiralis namely, 6400 larvae per os, and cortisone on the mortality rate of mice, and found that the average survival time of the cortisone treated mice (25 mg./kg. body wt.) was 42.5 hours while that of the infected controls was 71.8 hours. Cortisone suppressed innate resistance to T. spiralis of Chinese hamsters and golden hamsters. Cortisone-treated animals harboured adult worms for a longer period than the controls (Ritterson, 1959; Ritterson and Concannon, 1968).

Cortisone significantly increases the susceptibility of worm-free mice to infections with Aspicularis tetraptera (Mathies, 1962).

Methyl prednisolone in massive doses rendered 6-9 weeks old dogs more susceptible to the establishment of Necator americanus (Miller, 1966).

Weinstein (1953) used cortisone to establish a greater number of Nippostrongylus muris worms in rats. Cortisone lowered the resistance of guinea pig to infection with the rat nematode, Nippostrongylus brasiliensis. Nearly 11 times more larvae completed the skin-lung migration in the cortisone treated animals than in the controls (Parker, 1961). Ogilvie (1965) showed that daily treatment of rats with prednisolone or betamethasone suppressed the development of acquired resistance to Nippostrongylus brasiliensis but did not

affect the development of larvae in the course of a subsequent infection.

In rats, the larvae of Nematospiroides dubius penetrate into the intestinal wall and become encapsulated there. The connective tissue capsule in these hosts is much thicker than that in mice. This capsule prevents the return of maturing larvae into the intestinal lumen and the larvae die. Cross (1960) found that cortisone suppressed this tissue reaction and that in treated rats the maturing larvae can penetrate through the capsule and return to the intestine. Cortisone was most effective when given on the day of infection.

In summary, cortisone and its analogs impair the resistance of mammalian hosts to at least the following helminths:

TREMATODA	Digenea	Paragonimus miyazakii	Tada (1967)
CESTODA	Cyclophyllidea	Taenia taeniaeformis	Oliver (1962)
		Taenia multiceps	Esch (1967)
		Hymenolepis nana	Weinman et al, 1961
NEMATODA	Trichuroidea	Trichinella spiralis	Coker (1956)
		Trichuris muris	Campbell (1963)
	Strongyloidea	Necator americanus	Miller (1966)
		Nippostrongylus muris	Weinstein (1955)
		N. brasiliensis	Parker (1961)
		Nematospiroides dubius	Cross (1962)
	Oxyuroidea	Aspicularis tetraptera	Mathies (1962)
	Filarioidea	Litomosoides carinii	Briggs (1963)

Therefore, it can be assumed that cortisone will depress the resistance of mice to larval Echinococcus multilocularis. In the present thesis, an attempt is made to study the influence of cortisone on the growth of implanted E. multilocularis cysts in rodents.

MATERIAL AND METHOD

1. Parasite and Hosts

The parasites used in the present research are the larvae of *Echinococcus multilocularis*.

This parasite was discovered by Rausch & Schiller (1951) on St. Lawrence Island, Alaska in 1950. Dr. R. L. Rausch mailed onchospheres from infected foxes to Dr. T. W. M. Cameron at the Institute of Parasitology, Macdonald College, Montreal (Lubinsky, 1960b). Dr. Lubinsky, using cotton rats infected with these onchospheres, established a vegetatively propogated strain in cotton rats in 1958. Fertile cysts from the 46th to 57th transfers in cotton rats were used as a source of infection of all my experimental animals.

The rodent hosts used belonged to a few species:

cotton rat	*Sigmodon hispidus*
gerbil	*Meriones unguiculatus*
white and hooded rat	*Rattus norvegicus*
laboratory mouse	*Mus musculus*
strains	CBA
hybrids	LD_1F_1 (cross between C57L female and DBA_1 male)
	$B6D2F_1$ (cross between C57Bl/6 female and DBA_2 male)

Cotton rats used in my experiments were the progeny of wild cotton rats from Arizona, U.S.A. Gerbils were descendants of animals purchased from the Tumblebrook Farm, Inc., Brant Lake, U.S.A. Laboratory mice were obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbour, Maine, U.S.A.

Cotton rats were housed in large cages with metallic perforated lids and with wood shavings as bedding. The mice were kept in cages of South Illinois University type purchased from Canlab.

Each cage has a disposable plastic bottom, a metal stand, a perforated lid and a pellet hopper. With wood shavings as bedding, each cage provides a floor surface of about 48 square inches. The animal rooms were located in the basement, Buller Building, University of Manitoba. The lighting simulated daylight cycle, being turned on at 6:00 a.m. and off at 10:30 p.m. The room temperature varied between 70-78°F.

All the rodents were given Purina lab chow and water ad libitum. The normal water consumption was estimated to be 5 ml./mouse/day for brown LD_1F_1 mice and a little higher for the black $B6D2F_1$ hybrids.

Animals used in experiments were identified by ear clippings. Saturated aqueous solution of picric acid was occasionally used to stain hooded rats for the same purpose.

The age of animals indicated in the tables is the age at the time of infection.

2. Infection with vegetatively propogated larval Echinococcus multilocularis

This technique is similar to that used for the maintenance of solid tumors by serial transfers. The equipment used is shown in Fig. 6.

Donor cotton rats with 45 to 75 day old Echinococcus cysts were killed with chloroform and mounted on a dissection board. The ventral surface was shaved and disinfected with 70% ethanol. The viscera were exposed by a longitudinal cut from the sternum to the pubis. The abdominal wall was reflected. Clear intraperitoneal fertile cysts were selected and removed to sterile physilogical saline (0.85% w/V NaCl solution) in a Petri dish. Care was taken to

avoid perforation of the intestine. Penicillin G (Crystapen, Glaxo-allenburg, Canada) was added to inhibit bacterial growth.

Non-necrotic and non-hemorrhagic portions of cyst clusters were ground against an autoclaved wire mesh (7 mesh per cm) to yield cyst fragments. Intact protoscoleces were present in the material which passed through the mesh. The cyst material was diluted with a calculated volume of sterile physiological saline. Penicillin G was added to provide a concentration of 100 to 1000 I.U./ml. of the final suspension.

To provide a criterion for the amount of cyst material used to infect animals in different experiments, a concentration index was used. This is defined as the ratio

$$\frac{\text{cyst material in c.c.}}{\text{cyst material in c.c + saline in c.c.}}$$

In practice, it was assumed that the specific gravity of the cyst material is close to 1.0. The concentration index permits us to find out the volume of undiluted cyst material injected into each animal. The required volume equals the volume of diluted suspension injected multiplied by the concentration index.

In my experiments the concentration index varied from 0.17 to 0.53. It depended on the availability of good, clear alveolar cysts material and the number of experimental animals to be infected.

Intraperitoneal infections were made with a $\frac{1}{2}$-inch gauge 18 needle. The volume injected was:

laboratory mouse	0.5 ml.
gerbil	1.0 ml.
white and hooded rat	1.5 ml.

Figure 6

Equipment used to infect rodents with the vegetatively propogated strain of *Echinococcus multilocularis*

Figure 7

Plastic rodent immobilizer

The apparatus facilitates intravenous injection and collection of peripheral blood from the caudal lateral veins. The anterior chamber contains cotton soaked in ether to anaethetize the animals.

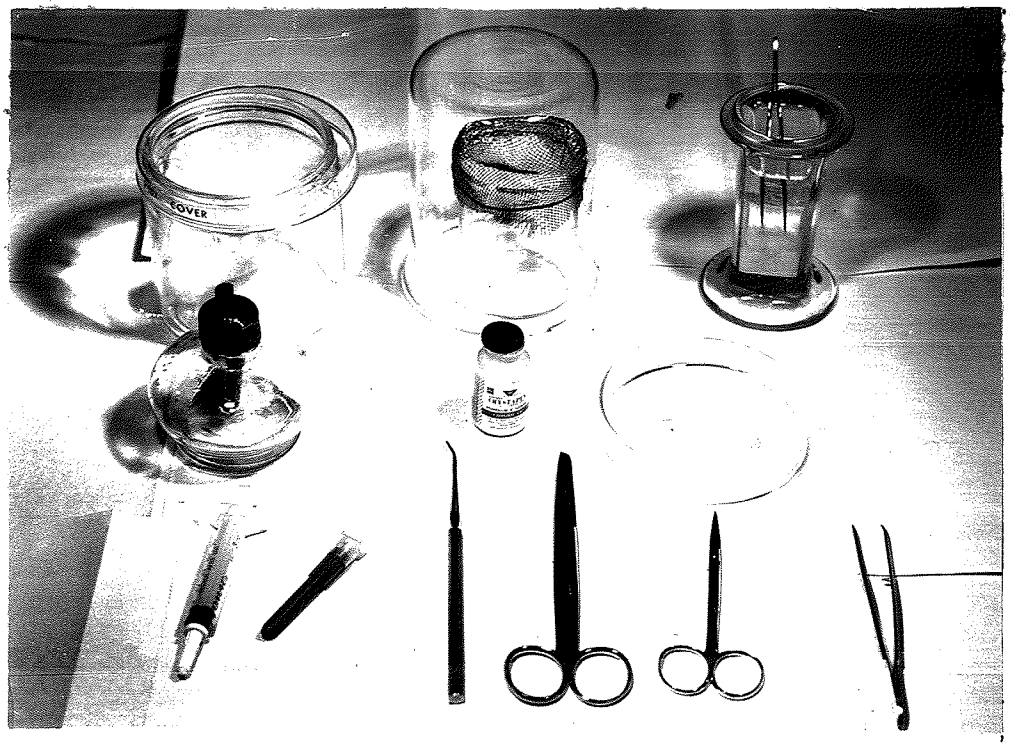


Figure 6

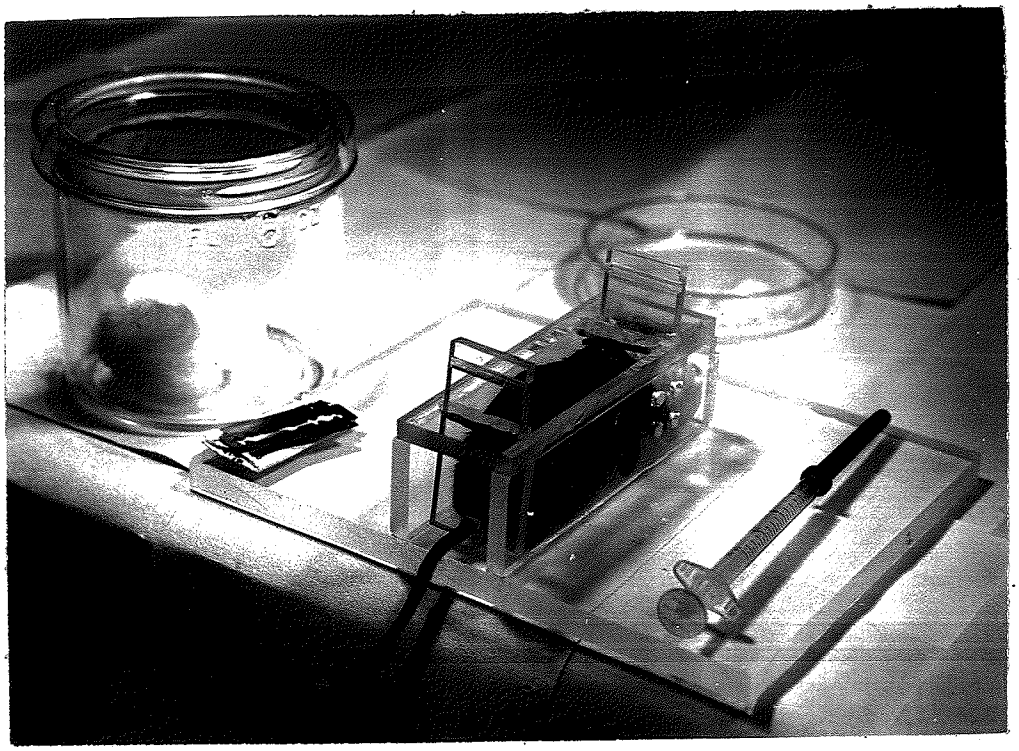


Figure 7

Some degree of uniformity of the inoculum (cyst suspension) was achieved by repeatedly aspirating and ejecting the ground material with a syringe.

In chemotherapy experiments, Penicillin G was not used to avoid complications in drug actions.

3. Carbon Particle Blockade

In my experiments, I used Indian ink to block the reticulo-endothelial system prior to infection.

Two kinds of Pelikan Indian ink were used. The first kind which contained 80 mg. carbon/ml. with shellac was used in the early experiments. The size of its carbon particles was about 20-50 mμ. The second kind, C11/1431a, Günther Wagner, contained about 80-100 mg. carbon/ml. suspended in a solution of fish glue with some phenol as preservative, but without shellac. The size of carbon particles was similar to that of the first kind (Halpern et al, 1953). The ink was diluted with physiological saline to the concentration required.

Two routes of injections were used: the intraperitoneal and the intravenous. Both routes were satisfactory in achieving the "blockade"; the darkening of pinnae and feet several minutes after injection indicated the entry of carbon particles into the circulation.

The intravenous injections into the tail veins were fascilitated by the use of an acrylic plastic immobilizer (Fig. 7). This material is insoluble in ether, but soluble in xylol and

chloroform. The mouse was lightly anaesthetized with ether and put into the immobilizer with the trap-door closed. Cotton swabs soaked in ether were placed into the anterior chamber to maintain the anaesthesia. The tail was put into the indentation of the trapdoor, disinfected with 70% ethanol, dried with cotton wool and held taut. A half inch, gauge $27\frac{1}{2}$ needle connected to a tuberculin syringe was used to make injections into one of the lateral caudal veins. The volume was limited to 0.10 ml. per 10 gm. body weight. Vasodilation caused by ether anaesthesia was convenient for the venipuncture. Other means of causing local vasodilation such as warming the tail under a lamp or in warm water or rubbing it with xylol were not necessary. The actual injection takes 3 to 7 seconds. After repeated injections, the veins become difficult to find. Subsequent injections become more difficult.

The first injection of ink was given 5 days before infection. The total dose of carbon used ranged from 300 to 2000 mg./kg. body weight.

4. Splenectomy

Animals for splenectomy experiments were of matched age, sex and body weights. Two days before operation, they were transferred into new cages, five per cage, and allowed to settle.

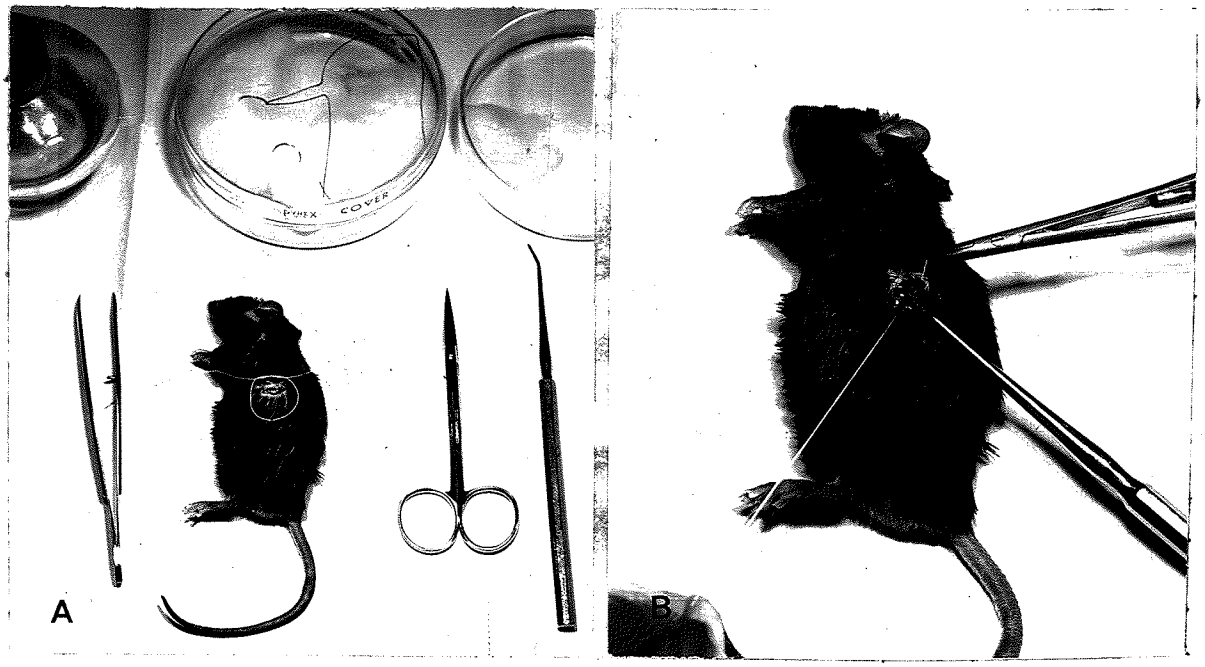
Immediately before the operation, the animal was anesthetized with ether and was injected intraperitoneally with 1% Avertin (tribromoethanol, Winthrop) aqueous solution prewarmed to 40°C. The dose of Avertin for different animals was:

Mouse	100 mg/kg body weight
Gerbil	50 mg/kg body weight
Hooded Rats	40 mg/kg body weight

Figure 8

Techniques of splenectomy

A. Incision in the anterior, left side of abdominal wall; B. ligature of splenic vessels; C. suturing the musculature: D. suturing the skin.



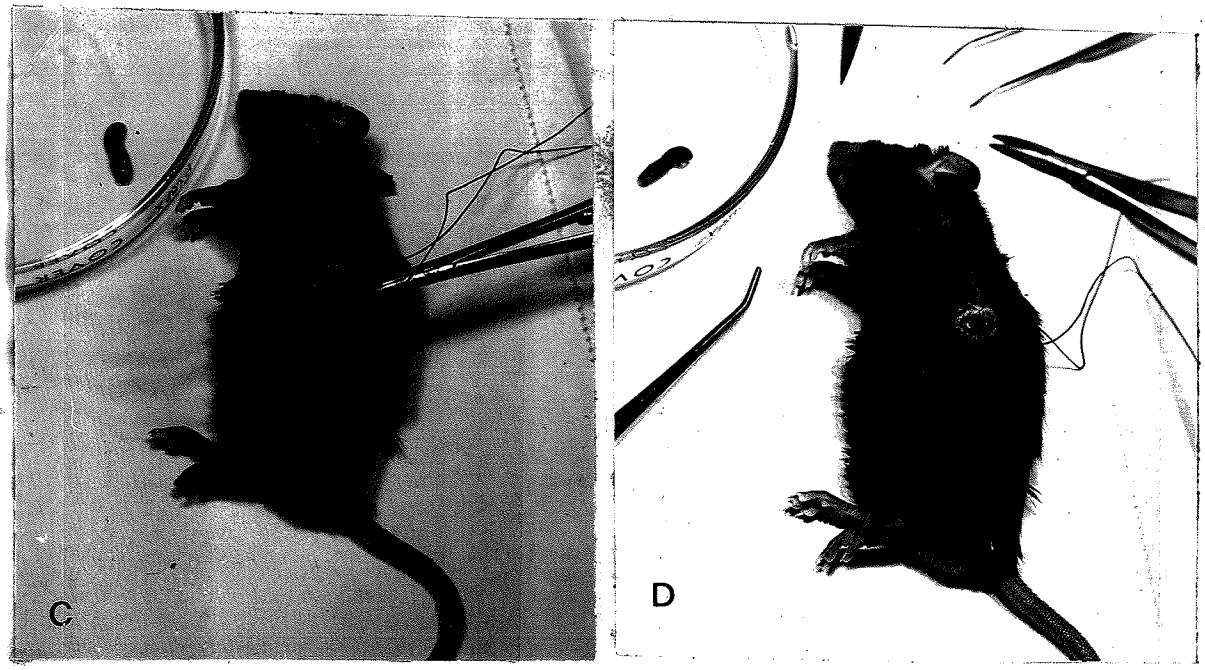


Figure 8

The narcosis lasted for at least 20 minutes. The passing of anesthesia is shown by the following phenomena: increased abdominal and thoracic respiration, slight squeaking and shivering, sitting up or staggering around.

The operation was carried out in an operating room under aseptic conditions. The anesthetized animal was placed on its right side (Fig. 8). The left side was rubbed with 70% ethanol. In mice and gerbils a transverse 1 cm. long dorso-ventral incision through the skin was made at a distance of 0.5 cm. posterior to the last rib. In hooded rats, the incision was 2 cm. long and made 2 cm. posterior to the last rib. A smaller incision was made through the musculature. The spleen was cut off, the splenic vessels were ligated and the mesenteric stump was pushed back into the abdominal cavity. The incision of the musculature was closed with one stitch and that of the skin with two stitches. Round bodied, half inch circle No. 18 needles and black braided surgical silk, Ethicon, 000 were used. With the help of an experienced assistant, the entire operation took only four minutes. All animals to be used on one experiment were splenectomized within the same day.

A preliminary test for latent infection with Bartonella muris was made on two hooded rats and two gerbils. The peripheral blood of splenectomized animals remained negative for Bartonella for at least 12 days.

In most of my experiments, splenectomy was performed three days before infection. The splenectomized animals were infected with Echinococcus multilocularis cysts by injecting the material into the right side of the abdominal cavity.

5. Cortisone treatment

Only LD_1F_1 mice were used in these experiments. Under light ether anesthesia, each mouse received a subcutaneous injection of cortisone (cortone acetate, Merck Sharp & Dohme, Canada) suspension in physiological saline. The injection site is the dorsal lumbar region. One tenth of a ml. of the suspension per ten grams of body weight was injected with a gauge 20-24 needle using a 1 ml. tuberculin syringe.

6. Haematological techniques

Three techniques were used: (a) collection of peripheral blood, (b) staining blood smears for differential leucocyte counts and (c) total leucocyte counts using a counting chamber.

(a) Collection of peripheral venous blood

Blood was taken from the lateral caudal veins. Orbital bleeding technique by rupturing the fragile opthalmic venus plexus (Riley, 1960) which imposes a psychological stress on the bleeder was not used. To avoid undue effects, each infected mouse was bled only once just before autopsy. The bleeding was done between noon and 4:00 p.m. Under light ether anesthesia, the mouse was put into the plastic rodent immobilizer (Fig. 7) with the trap door closed. The tail was disinfected with 70% ethanol and wiped dry with cotton. A lateral vein was severed with a razor blade to allow free flow of blood. The first drop of blood was discarded. Thin smears were prepared on grease free slides. For total leucocyte count, the blood was collected into WBC pipettes using a suction apparatus with an air-tight gasket (No. A-2473, Clay-Adams, U.S.A.).

(b) Differential leucocyte count

For the preparation of good thin blood films, the best diameter of the blood drop was found to be about 3 mm.; the best angle between the spreader and the smear slide as 30° or smaller. Smears were stained with Wright's stain using McIlvaine's buffer solution at a pH of 6.7 (Lubinsky, 1960a). Two hundred leucocytes were counted in each slide under oil-immersion at 1000X and classified as lymphocytes, monocytes, neutrophils, eosinophils and basophils. For simplicity, the relatively uncommon metamyelocytes (juveniles) and band-nucleus neutrophils (ring-nucleus neutrophils, staffs and stabs) were grouped together with the mature neutrophils (Diggs, Sturm and Bell, 1956).

(c) Total leucocyte count

Fresh blood was drawn to the 0.5 mark of a white blood cell pipette. The blood was diluted 1:20 with Darmady and Davenport's diluting fluid for total leucocyte count (glacial acetic acid, 2 ml.; distilled water, 98 ml.; a 1% aqueous solution of gentian violet; 1 ml.). The suspension was mixed thoroughly using a pipette shaker. The 5th and the 9th drop of the suspension were emptied into the two counting chambers of a Spencer Double Improved Neubauer Haemocytometer. The cells were allowed to settle for 3 minutes, and counted at 100X. The leucocytes in five large squares of each chamber of the hemocytometer were counted. In case of high leucocytosis, a minimum of 500 leucocytes per chamber was counted. The area of each large square is 1 $mm.^2$. The depth of the chamber is 0.1 mm.

7. Photography

Asahi Pentax Spotmatic camera with a built-in light meter and 55 mm. f/1.8 lens was used to take 35 mm. pictures. Extension tubes were used for close-ups up to a magnification of 1.17 X. Enlargements were made on Kodak Polycontrast F paper and projection slides were made by contact printing on a blue-sensitive film (Kodak Fine Grain Positive Film). Kodak High Contrast film was used to take pictures of line drawings and projection slides were made from these negatives by contact printing on Kodak Orthochromatic film.

PLANS OF INVESTIGATION

In the present thesis, an attempt is made to answer 3 questions.

a. Does the blockade of the RES influence the growth of Echinococcus cysts?

b. Does the splenectomy affect the growth of Echinococcus cysts?

c. Does the administration of cortisone accelerate the growth of Echinococcus cysts?

Group means were obtained for all measurements and standard error of the means calculated. Student's t test was used for statistical analyses. Probability levels greater than 5 percent were considered as having no statistical significance.

RESULTS

1. The blockade of the RES with Indian ink

Only male mice were used in these experiments. A total dose of 300 mg. of colloidal carbon per kg. body weight was used as the minimum blocking dose (Table II). Four experiments were made.

Experiment 1 Three groups of 5-month old LD_1F_1 mice were infected intraperitoneally with Echinococcus cysts from a cotton rat donor. Five days before infection, the experimental mice received intraperitoneal injections of diluted Pelikan Indian ink. Mice of the first experimental group got 333 mg. carbon per kg. body weight, and the second 1000 mg./kg. All the mice were autopsied at 60 DPI. The difference in cyst weight between the blockaded animals and the controls was not significant. Contrary to expectation the second experimental group which received 1000 mg./kg. of carbon, had the lowest cyst weight.

Experiment 2 This experiment almost duplicated the first one, but had a third experimental group of blocked mice that received a total of 2000 mg. carbon per kg. body weight. Seven out of ten mice of this group died within ten days after infection. The autopsy of all mice at 76 DPI showed that even when the ink was given in a sublethal dose, the difference in the weight of the cysts between the experimental mice and the controls was not statistically significant. In ink-treated mice a hypertrophy of the patches of Peyer was frequently observed in the distal part of the small intestine. Paradoxically, a negative correlation seemed

Table II

Growth of Larval Echinococcus multilocularis in Ink-blockaded Male Mice

Experiment	Type of Mice	age months	Group	Carbon Dosage Pre-infection mg./Kg	Carbon Dosage Post-infection mg./Kg.	Route	Concentration Index *	Survival Ratio	Autopsy DPI**	Body Wt. before Treatment gm.	Gross Wt. at Autopsy gm.	Spleen Wt. gm.	Cyst Wt. gm.	t	p
1	LD_1F_1	5	Control	-	-	-	0.17	17/20	60		32.7	0.17±0.02	0.86±0.22		
			Exp.#1	333x 1	-	i.p.	0.17	12/25	60		32.6	0.21±0.03	1.65±0.49	1.62	>0.10
			Exp.#2	333x 3	-	i.p.	0.17	11/16	60		33.8	0.14±0.02	0.30±0.14	1.87	>0.05
2	LD_1F_1	6	Control	-	-	-	0.17	21/25	76	31.0	34.4	0.36±0.04	4.28±0.66		
			Exp.#1	333x 1	-	i.p.	0.17	22/27	76	30.3	34.9	0.39±0.05	4.97±0.73	0.70	>0.10
			Exp.#2	333x 3	-	i.p.	0.17	21/27	76	30.2	34.4	0.25±0.04	3.50±1.15	0.59	>0.10
			Exp.#3	1000x 2	-	i.p.	0.17	3/10	76	29.7	32.7	0.09±0.00	0	significant	
3	CBA	7	Control	-	-	-	0.53	22/24	117	30.6	33.3	0.16±0.02	1.85±0.41		
			Exp.	333x 1	-	i.p.	0.53	22/24	117	30.6	34.5	0.16±0.02	1.68±0.57	0.24	>0.10
4	LD_1F_1	2½	Control	-	-	-	0.39	12/15	31	25.6	33.2	0.54±0.02	11.91±0.42		
			Exp.#1	100x 3	-	i.v.	0.39	9/ 9	31	25.1	32.8	0.50±0.03	11.75±0.48	0.25	>0.10
			Exp.#2	100x 3	100x 1 (21 DPI)	i.v.	0.39	7/ 9	31	25.1	31.8	0.43±0.04	11.72±0.69	0.24	>0.10

* a measure of a dose of cyst material used for infecting mice.

** days post-infection.

to exist between the dose of ink and the weight of the cysts; mice which survived a total dose of 2000 mg./kg. had no cysts at all. The difference is not statistically significant.

Experiment 3 The possibility of increasing the growth rate of Echinococcus cysts in relatively resistant hosts by Indian ink blockade was investigated. Agouti CBA mice, 7 months old were used in the experiment (Fig. 3) Again, the difference in the cyst weight between the experimental and the control mice was not significant.

Experiment 4 This experiment was conducted to find out whether intravenous injections of Indian ink will be more efficient than the intraperitoneal ones in influencing the growth of Echinococcus cysts. Pelikan Indian ink (C11/143| a) which did not contain shellac were injected intravenously into young LD_1F_1 mice with a total dose of 300 mg. carbon per kg. body weight one to five days before infection. The first experimental group did not receive any more injections, but the second treated group received one injection of 100 mg./kg. at 21 DPI. The autopsy at 31 DPI showed that the difference in cyst weight between the blockaded mice and the controls was not statistically significant.

Thus all the experiments with the blockade of the RES with Indian ink consistently gave negative results.

2. Splenectomy

To study the role of the spleen in Echinococcus infections, three series of experiments were made. In all of them, the spleen

was removed before infection. The three series are:

(a) preliminary experiments with various rodents;

(b) protracted experiments with LD_1F_1 mice;

(c) protracted experiments with $B6D2F_1$ mice.

The growth of Echinococcus cysts is much faster in LD_1F_1 mice than in $B6D2F_1$ mice (Fig. 3). It is fast in gerbils but very slow in hooded rats.

a. Observations on various rodents

(1) General observations

LD_1F_1 mice: The body weight of splenectomized mice dropped to a minimum of approximately 90% of the original weight, 2 to 3 days after operation. Then the gross weight of both controls and splenectomized mice increased. At autopsy the net body weight, i.e. the total weight minus cyst weight, in both groups was much lower than the original body weight just before infection.

Gerbils: The post-operative mortality of these rodents was high, probably due to imperfect surgical techniques. The net body weight of gerbils at autopsy was much lower than that before infection.

Hooded rats: The post-operative mortality of these rats was high. The net body weight of the rats at autopsy was much higher than that before infection.

(2) Growth of Echinococcus cysts in splenectomized rodents (Table III)

Experiment 1 Forty-six LD_1F_1 male mice were used, 25 of them splenectomized. All the mice were dissected at 45 DPI. The cyst weight in splenectomized mice was 10.24 ± 0.73 gm and that in the controls is 9.36 ± 0.88 gm., but the difference was not statistically significant.

Table Ⅲ

Growth of larval Echinococcus multilocularis in splenectomized rodents

Experiment No.	Rodent	Age month	Sex	Group	Splenectomy DPI	Survival ratio	Autopsy DPI*	Body wt. before treatment gm.	Gross wt. at autopsy gm.	Spleen wt. gm.	Cyst wt. gm.	t	p
1	Mice (LD_1F_1)	2½	♂	Control	-	16/21	45	26.1	29.0	0.31± 0.11	9.36± 0.88		
				Exp.	-4	17/25	45	25.8	28.5	-	10.24± 0.73	0.67	>0.10
2	Mice (LD_1F_1)	4	♀	Control	-	25/25	30	23.3	25.4	0.47± 0.03	3.25± 0.17		
				Exp.	-3	27/29	30	23.3	24.3	-	3.13± 0.12	0.59	>0.10
3	Gerbil	2	♂	Control	-	5/ 8	41	65.4	63.9		18.26± 1.98		
				Exp.	-3	3/ 8	41	78.9	71.0		17.69± 1.44	0.20	>0.10
	Gerbil	2	♀	Control	-	6/ 8	41	57.8	59.0		18.40± 0.82		
				Exp.	-3	3/ 9	41	72.5	57.3		15.83± 0.97	1.90	>0.05
4	Hooded rat	2	♂	Control	-	8/ 8	203	252	392± 13	1.36± 0.11	26.53± 9.07		
				Exp.	-3	5/ 9	203	219	414± 24	-	24.85± 8.76	0.12	>0.10
	Hooded rat	2	♀	Control	-	4/ 5	203	168	249± 12	0.94± 0.24	23.50± 7.12		
				Exp.	-3	3/ 6	203	184	272± 22	-	20.33±16.75	0.19	>0.10

* days post-infection.

Experiment 2 Fifty-four LD_1F_1 female mice were used, 29 of them splenectomized. The experiment was discontinued 30 days after infection to see whether the period of infection has any effect on the growth of cyst weight. The average weight of cysts in both groups awas almost the same: 3.25 gm. in the controls and 3.13 gm. in the splenectomized mice. The difference was not significant.

Experiment 3 A smaller number of gerbils were used. The experimental and control groups are composed of only 8 or 9 animals each. The gerbils were dissected at 41 DPI. Both in the male and the female gerbils, the cyst weight was slightly smaller in the splenectomized animals than in the controls. But the difference was not statistically significant.

Experiment 4 Seventeen males and 11 females hooded rats were used. The growth of Echinococcus cysts in these hosts is extremely slow, and the production of protoscoleces was limited. The great majority of cysts were degenerated. The hooded rats were autopsied 203 days after infection. Again the cyst weight in the splenectomized animals was slightly smaller than in the controls. The difference was not statistically significant.

b. Protracted experiments with LD_1F_1 mice

Two experiments were made, the first with male LD_1F_1 mice and the second with females. The results of these experiments are summarized in Tables IV and V.

(1) Experiment 1 The experiment lasted 62 days. A total of 185 male LD_1F_1 hybrids mice was divided into 3 groups:

Group I - 80 infected contols
Group II - 100 splenectomized, infected mice
Group III - 5 healthy, uninfected controls

Table IV

Growth of larval Echinococcus multilocularis in splenectomized LD_1F_1 mice

Group	Splenectomy DPI*	Concentration index +	Number autopsied	Autopsy DPI	Cyst Weight gm.	t	p
First Experiment : ♂♂ LD_1F_1 mice, 5-7 months old**							
Control	-	0.24	16	20	0.39± 0.07		
Exp.	-3	0.24	18	20	0.43± 0.07	0.42	>0.10
Control	-	0.24	13	29	0.97± 0.19		
Exp.	-3	0.24	18	29	1.04± 0.20	0.25	>0.10
Control	-	0.24	14	43	4.29± 0.68		
Exp.	-3	0.24	15	43	4.33± 0.69	0.05	>0.10
Control	-	0.24	14	54	6.00± 1.66		
Exp.	-3	0.24	15	54	5.50± 0.81	0.28	>0.10
Control	-	0.24	10	62	6.59± 1.76		
Exp.	-3	0.24	11	62	10.05± 1.26	1.62	>0.10
Second Experiment : ♀♀ LD_1F_1 mice, 2-3 months old **							
Control	-	0.22	16	39	0.53± 0.12		
Exp.	-2	0.22	19	39	0.43± 0.13	0.56	>0.10
Control	-	0.22	12	77	4.07± 1.14		
Exp.	-2	0.22	24	77	5.73± 1.21	0.72	>0.10

* days post-infection

** at the time of infection

\+ a measure of a dose of cyst material used to infect mice

Table V

Host responses of splenectomized LD_1F_1 mice

	DPI	Group I Infected Controls		Group II Splenectomized Infected		Group III Healthy Controls
First Experiment :	LD_1F_1 ♂♂					
Gross Body Wt.	-4	28.9	(80)*	29.1	(100)	
	20	28.4	(16)	27.5	(18)	29.4(2)
in gm.	29	29.3	(13)	28.1	(18)	-
	43	30.0	(14)	29.7	(15)	-
	54	30.9	(14)	30.3	(15)	-
	62	31.5	(10)	32.4	(11)	30.6(3)
Spleen Wt.	20	0.31± 0.03	(16)	-		0.08 (3)
	29	0.32± 0.04	(13)	-		-
in gm.	43	0.42± 0.05	(14)	-		-
	54	0.36± 0.03	(14)	-		-
	62	0.36± 0.04	(10)	-		0.07 (3)
Second Experiment :	LD_1F_1 ♀♀					
Gross Body Wt.	-3	21.0± 0.3	(32)	21.0± 0.3	(46)	
in gm.	39	23.3± 0.4	(16)	22.5± 0.3	(19)	20.8± 0.4(9)
	77	24.2± 0.7	(12)	26.3± 0.6	(24)	23.3± 0.6(9)
Spleen Wt.	39	0.21± 0.03	(16)	-		0.07
in gm.	77	0.27± 0.05	(12)	-		0.07
Kidney Wt.	39	0.18± 0.02	(16)	0.18± 0.00	(24)	0.20
in gm.	77	0.19± 0.01	(12)	0.20± 0.01	(24)	0.20
Total WBC	39	18,670± 3,640	(15)	25,850± 3,100	(16)	9,880± 840(9)
cells/mm^3	77	34,150±12,150	(10)	47,760± 5,700	(22)	9,590±1,210(9)

* Number in bracket indicates the number of mice used.

The 100 splenectomized mice and the 80 controls were infected with Echinococcus cysts from a cotton rat with the 54th transfer of cysts. The cysts were 69 days old.

In this experiment, groups of 11 to 18 splenectomized mice and an approximately equal number of controls were dissected at 5 different time intervals, i.e. 20, 29, 43, 54 and 62 days post-infection (DPI).

The growth of Echinococcus cysts At all time intervals except the last one, the average cyst weight in splenectomized animals (Group II) was close to that in the controls (Group I). The small differences were not statistically significant (Table IV). At the final autopsy two months after infection, the average cyst weight in the splenectomized mice was 10.05 $\pm$ 1.26 gm. and in the controls 6.59 $\pm$ 1.76 gm. Even this big difference was not statistically significant. The t value was 1.62. This still may indicate a real difference in cyst weight.

The growth curves of the parasite in terms of cyst weight in both Group I and Group II mice were basically sigmoid. They ran close to each other until 54 DPI (Fig. 9). The first phase (slow growth) lasted about 20 days. The second phase (acceleration) occurred between 20 to 29 DPI. Then a phase of very fast, almost rectilinear growth set in. This stage continued to the final autopsy at 62 DPI. The weight of Echinococcus cysts in individual mice varied greatly. This is reflected in the large standard errors of the means. The cysts of the controls did not differ from those of splenectomized mice in microscopic appearance and in the distribution in the abdominal cavity. No protoscoleces were found in either groups.

Figure 9

Growth of larval Echinococcus multilocularis in LD_1F_1 male mice splenectomized 3 days prior to infection.

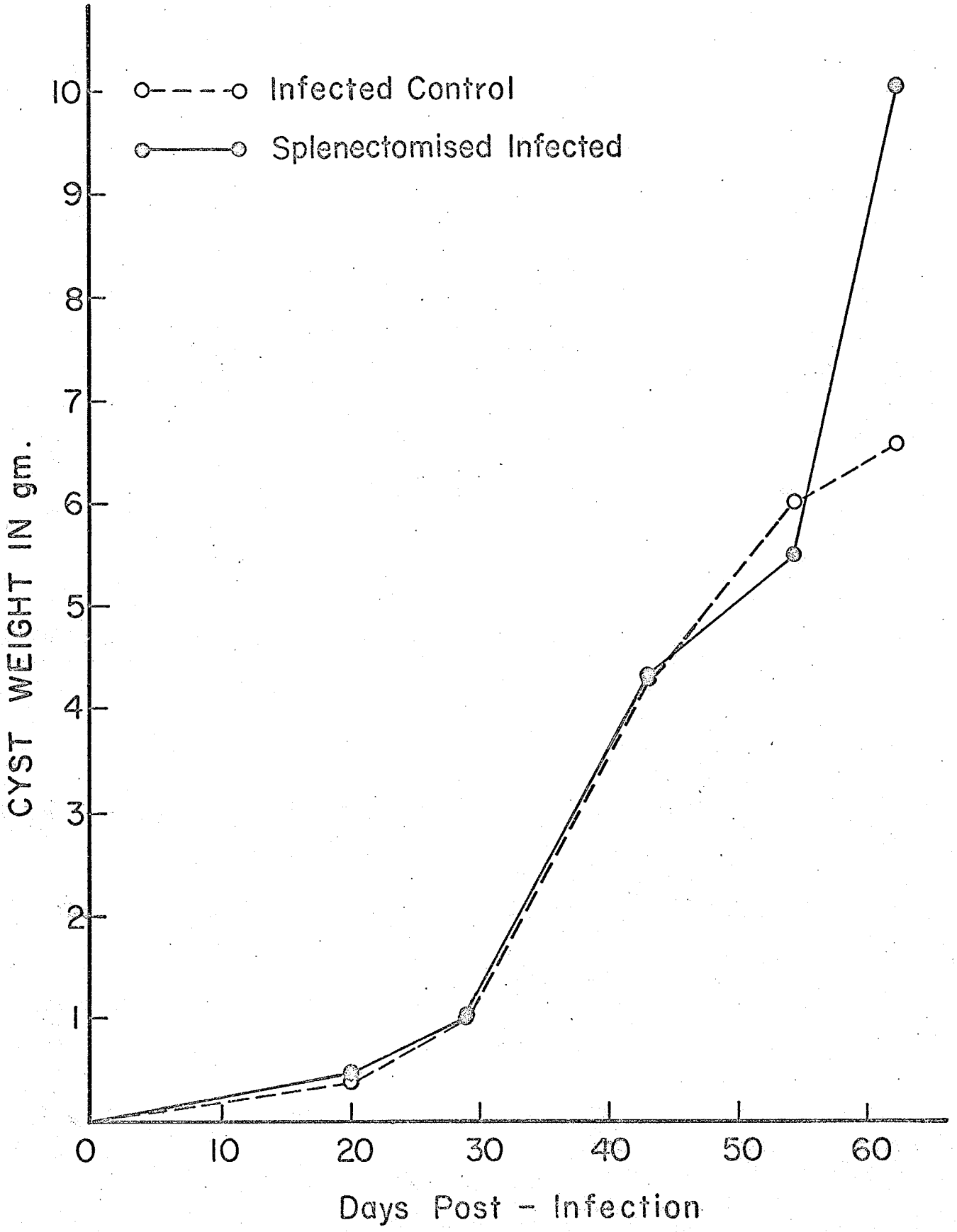

Infected Control
Splenectomised Infected
CYST WEIGHT IN gm.
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
Days Post - Infection

Figure 9

Figure 10

Mortality rate of splenectomized LD_1F_1 male mice infected with *Echinococcus multilocularis*

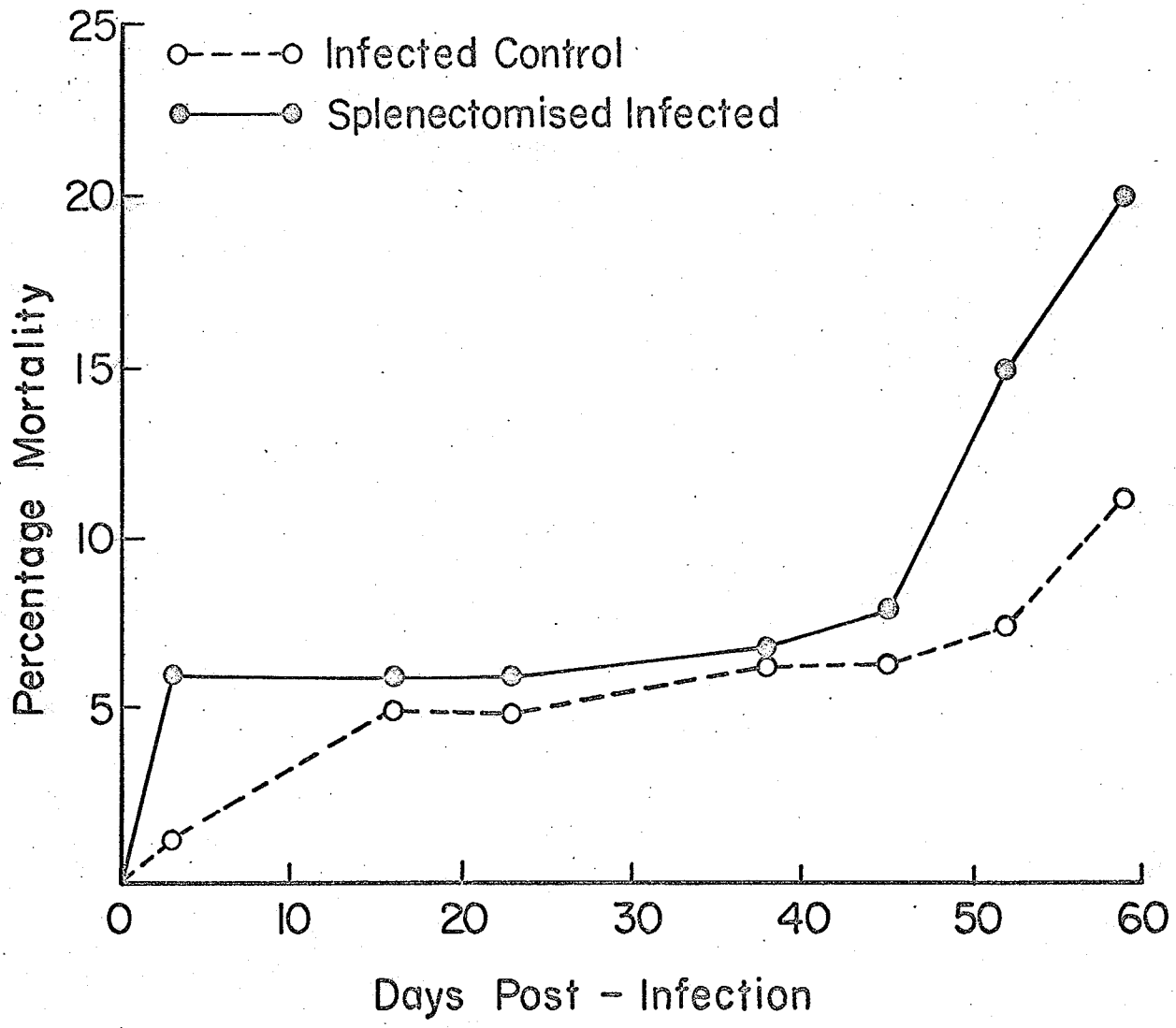

Infected Control
Splenectomised Infected
Percentage Mortality
25
20
15
10
5
0
10
20
30
40
50
60
Days Post - Infection

Figure 10

Figure 11

Host responses of splenectomized LD_1F_1 male mice A. "Net" body weight - gross weight minus weight of Echinococcus cysts: B. Spleen weight at autopsies.

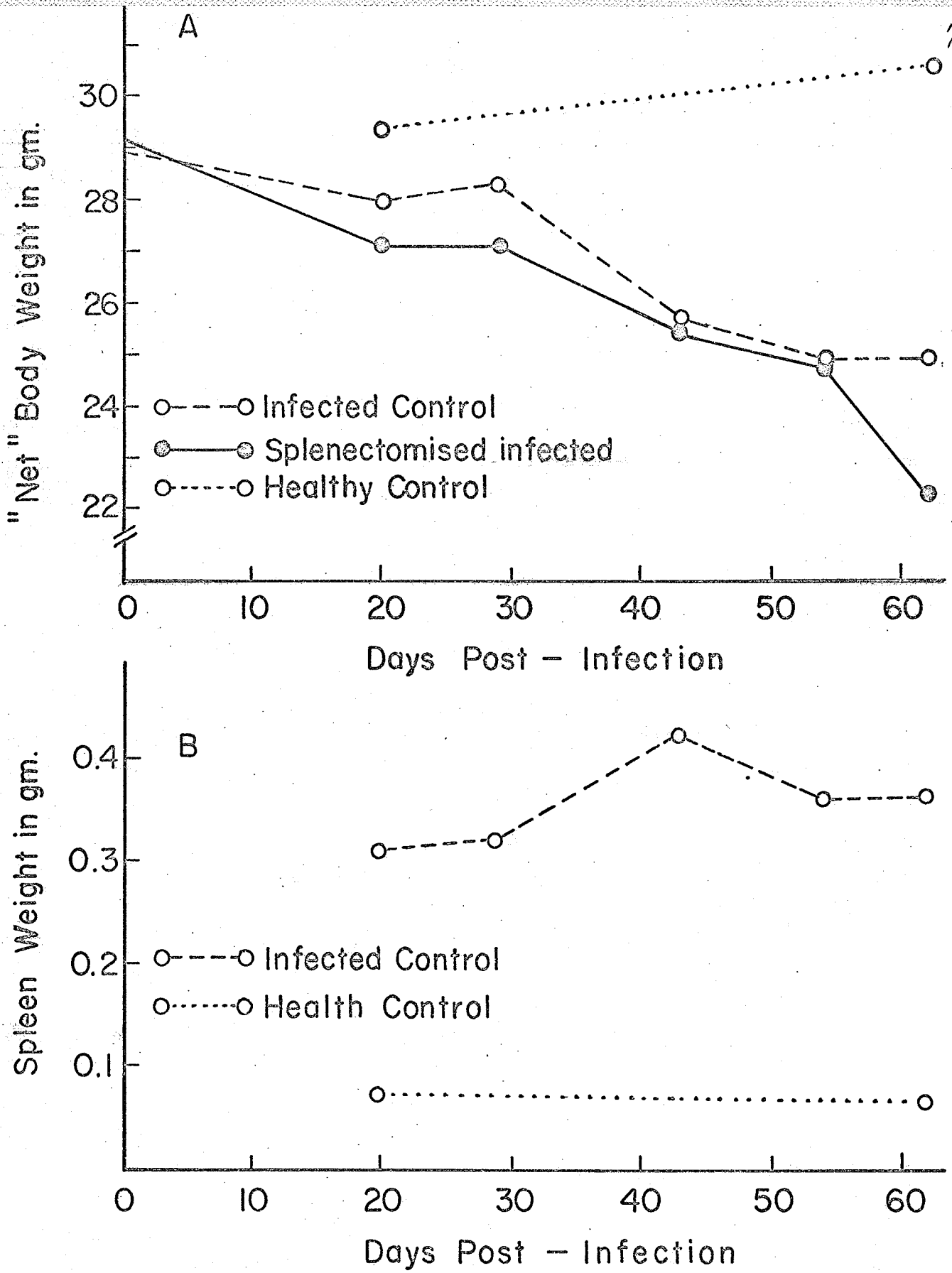

A
"Net" Body Weight in gm.
30
28
26
24
22
Infected Control
Splenectomised infected
Healthy Control
0
10
20
30
40
50
60
Days Post – Infection
B
Spleen Weight in gm.
0.4
0.3
0.2
0.1
Infected Control
Health Control
0
10
20
30
40
50
60
Days Post – Infection

Figure 11

Figure 12

Splenomegaly in LD_1F_1 male mice infected with *Echinococcus* cysts

The upper row shows the spleens of infected mice at 43 DPI. The lower row shows the spleens of normal controls.

This was expected because the production of protoscoleces in LD_1F_1 mice starts after three months.

Responses of the host The mortality rate of the host is expressed as cumulative death toll in percentage (Fig. 10). Before 45 DPI, there was no significant difference in this toll between splenectomized mice and the controls. However, the mortality rates diverged 43 days after infection. At 59 DPI, the splenectomized mice reached 20% compared to 11.3% of the infected controls. The net body weight in all infected mice dropped continuously. The average weight of infected controls decreased from 28.9 gm. 4 days before infection to 24.9 gm 62 days after infection; that of the splenectomized, infected mice from 29.1 gm to 22.3 gm. In the same period, the healthy uninfected controls (Group III) gained in weight (Fig. 11A). The average weight of the spleen in a healthy mouse is about 0.07 gm (Fig. 11B). The infected controls (Group I) at first developed splenomegaly which reached a peak 43 days after infection and then gradually subsided (Fig. 11B). This maximum average splenic weight was 0.42 gm. Later this weight decreased slightly and between 50 DPI and the end of the experiment at 62 DPI, the splenic weight remained constant.

(2) Experiment 2 This experiment was designed to study the difference in cyst weight in the late stages of infection. A total of 87 female LD_1F_1 mice was divided into three groups (Tables IV and V):

Group I - 32 infected controls
Group II - 46 splenectomized, infected mice
Group III - 9 healthy controls

Splenectomized mice and intact controls (Group I) were infected with Echinococcus cysts from the 55th transfer of cyst in cotton rat. The cysts used for infection were 71 days old. To prolong the survival time of the LD_1F_1 mice, a smaller inoculum was used. The infected mice were dissected 39 and 77 days after infection.

The growth of the Echinococcus cysts :- At 39 DPI, the average cyst weightin the control (Group I) was nearly the same as the splenectomized mice (Group II). At 77 DPI, the average weight of cysts in Group II was 5.73 $\pm$ 1.21 gm. and that of the controls 4.07$\pm$ 1.14 gm. Similar to the results observed in the first experiment with LD_1F_1 males, late in the course of infection, the cyst weight was much higher in the splenectomized mice than in the controls. Again the difference in cyst weight was not statistically significant. There was no difference in macroscopic appearance and distribution of cysts between Group I and Group II mice. No protoscoleces were found in either group.

Responses of the Host :- The mean net body weight of the infected controls (Group I) decreased from 21.0 gm 3 days before infection to 20.1 gm. at 77 DPI; that of the splenectomized, infected hybrids from 21.0 gm. to 20.6 gm. The healthy, uninfected controls gained 2.5 gm. per mouse between 39 DPI to 77 DPI. The splenic weight in infected controls (Group I) at 39 DPI was only slightly higher than that at 77 DPI. Neither the infection nor the splenectomy affected the weight of the left kidney.

The normal female LD_1F_1 mice had a total leucocyte count of about 9,730 cells per cu. mm. in the blood. The figure is higher than 8,600 $\pm$ 1,600 cells /cu. mm. in DBA/1 mice (Russell and Bernstein, 1966). This strain is one of the parental strains of LD_1F_1 hybrid. At 39 DPI and 77 DPI, the splenectomized infected hybrids (Group II) had a higher total leucocyte count than the infected controls (Group I) (See Table V). These findings agree with the observations of Ford (1968) who found that splenectomized rats developed leucocytosis.

C Protracted Experiment with male $B6D2F_1$ hybrid mice

In the third series of splenectomy experiments black $B6D2F_1$ males were autopsied at 4 time intervals; 31, 71, 110, 131 days after infection. A total of 186 male $B6D2F_1$ hybrids were divided into 4 groups:

Group I - 66 infected controls
Group II - 72 splenectomized, infected hybrids
Group III - 36 healthy, uninfected hybrids
Group IV - 12 splenectomized uninfected hybrids

The Echinococcus cysts grow much more slowly in the $B6D2F_1$ mice than in the LD_1F_1 (Fig. 3). For comparison 12 LD_1F_1 males, 6 months old, were infected simultaneously with the $B6D2F_1$ mice. The material used for infection was the 64 day old Echinococcus cysts in the 56th transfer of cysts in cotton rat. Each mouse received about 0.10 cc. of undiluted cyst material. All the $B6D2F_1$ mice used in this experiment were two months old at the time of infection. They were splenectomized 2 days before infection. The results are summarized in Tables VI and VII.

Table VI

Growth of larval Echinococcus multilocularis in splenectomized*B6D2F$_1$ ♂ mice**

Group	Concentration index +	Number autopsied	Autopsy DPI++	Spleen Wt. gm.	Cyst Weight gm.	t	p
Control	0.21	17	37	0.29± 0.03	0.32± 0.06		
Exp.	0.21	17	37	-	0.39± 0.08	0.76	>0.10
Control	0.21	16	71	0.30± 0.03	1.75± 0.50		
Exp.	0.21	12	71	-	2.29± 0.61	0.69	>0.10
LD_1F_1-control	0.21	11	70	0.32± 0.03	7.31± 2.39	2.71	<0.05
Control	0.21	12	110	0.31± 0.03	4.60± 0.80		
Exp.	0.21	11	110	-	3.09± 1.10	1.13	>0.10
Control	0.21	10	131	0.47± 0.10	3.29± 0.68		
Exp.	0.21	9	131	-	2.27± 0.83	0.94	>0.10

* splenectomized two days before infection
** two months old at the time of infection
\+ a measure of the dose of cyst material used for infecting mice
++ days post-infection

An additional group of LD_1F_1 ♂ mice was used to compare the degree of susceptibility to Echinococcus infection. These mice were 6½ months old at the time of infection.

Figure 13

Growth of larval *Echinococcus multilocularis* in $B6D2F_1$ male mice splenectomized 2 days prior to infection.

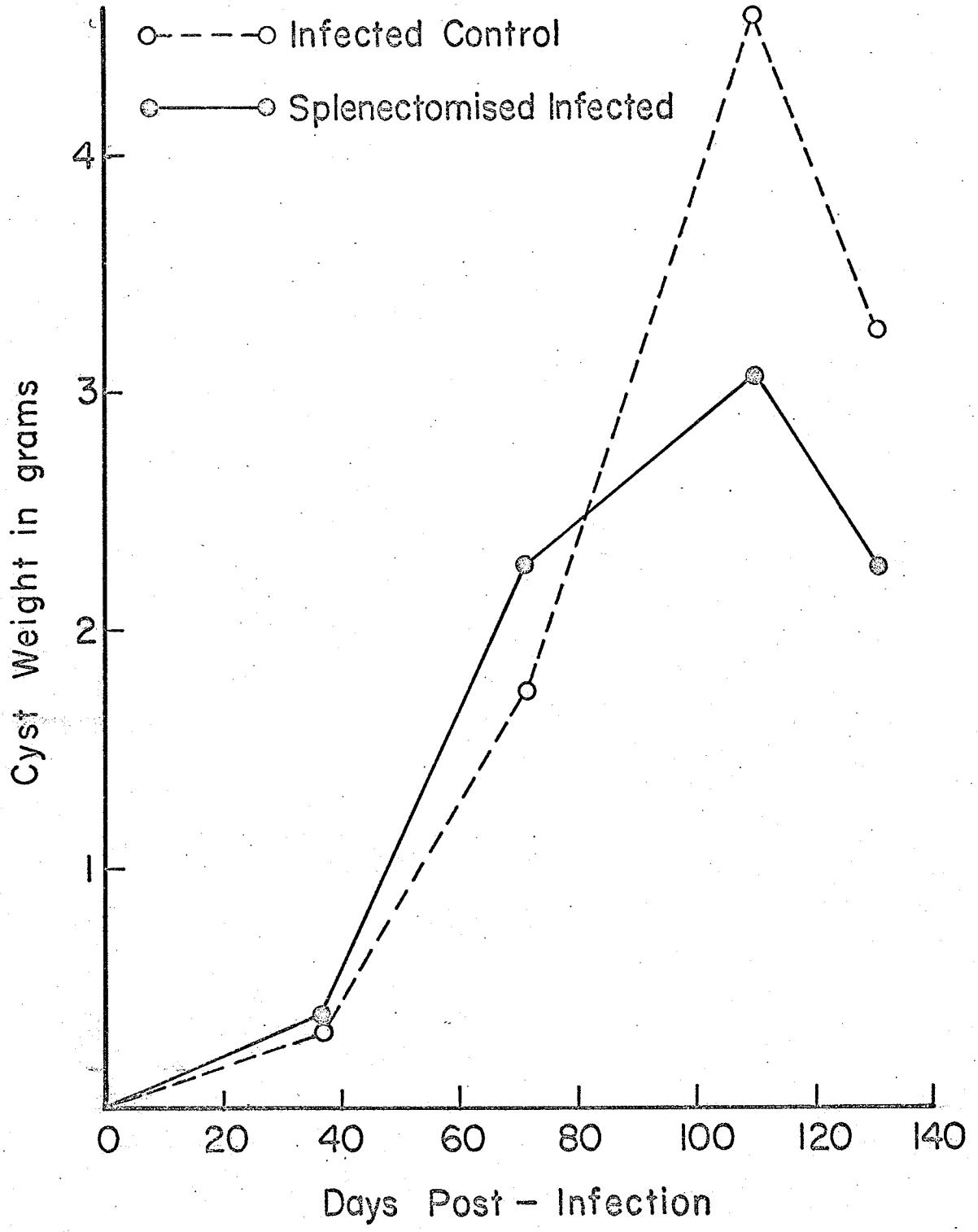

Infected Control
Splenectomised Infected
Cyst Weight in grams
1
2
3
4
0
20
40
60
80
100
120
140
Days Post – Infection

Figure 13

Table VII

Host Responses of Splenectomised $B6D2F_1$ ♂ Mice

	DPI *	Group I Infected Control		Group II Splenectomised Infected		Group III Healthy Control		Group IV Splenectomised Control	
Gross Body Wt. gm.	-3	21.6	(66)**	21.6	(72)	21.3	(36)	22.0	(12)
	37	25.4± 0.6	(17)	25.2± 0.7	(17)	26.9± 0.6	(8)	24.3± 0.9	(3)
	71	26.5± 1.0	(16)	24.6± 1.1	(12)	28.5± 0.6	(11)	30.2± 0.7	(4)
	110	27.8± 1.1	(12)	29.8± 0.7	(11)	30.6± 1.1	(7)	31.6± 0.8	(3)
	131	27.7± 1.7	(10)	27.7± 1.7	(9)	32.2± 0.8	(8)	30.6± 1.1	(4)
Spleen Wt. gm.	37	0.29± 0.03	(17)	-		0.07	(1)	-	
	71	0.30± 0.03	(16)	-		0.07	(1)	-	
	110	0.31± 0.03	(12)	-		0.07	(1)	-	
	131	0.47± 0.10	(10)	-		0.06	(1)	-	
Kidney Wt. gm.	37	0.20± 0.01	(17)	0.20± 0.01	(17)	0.20	(1)	0.22± 0.00	(2)
	71	0.22± 0.01	(15)	0.22± 0.01	(12)	0.22	(1)	0.27± 0.01	(4)
	110	0.22± 0.01	(12)	0.26± 0.01	(11)	0.20	(1)	0.27± 0.03	(2)
	131	0.23± 0.02	(10)	0.22± 0.02	(9)	0.18	(1)	0.25	(1)
Total WBC per mm³	37	28,200± 4,360	(16)	35,970± 5,480	(17)	9,510± 1,720	(8)	16,100± 3,210	(3)
	71	26,800± 3,360	(15)	39,460± 6,210	(12)	8,220± 850	(9)	10,770± 2,000	(4)
	110	31,960± 6,450	(11)	24,930± 4,140	(10)	7,590± 1,650	(6)	8,070± 2,900	(3)
	131	28,430± 6,410	(6)	14,700± 4,790	(8)	8,290± 1,470	(7)	10,010± 1,670	(4)

*Days post-infection

**Number in bracket indicates the number of mice used.

Growth of cysts The growth curves of Echinococcus cysts in the splenectomized B6D2F_1 hybrids (Group II) and in the infected controls (Group I) are essentially similar (Fig. 13). Both curves run close to each other until 80 DPI. The initial phase (slow growth) and the second phase (acceleration) lasted about 40 days. The weight of Echinococcus cysts reached its maximum around 111 DPI: an average of 4.60 gm. in the controls (Group I) and 3.09 gm. in the splenectomized mice (Group II). The difference is not significant (Table VI). In contrast to the results of experiments with LD_1F_1 mice, the growth of cysts in splenectomized B6D2F_1 mice in late stages of infection was slower than that in the controls. After reaching the peak values, both curves dropped off sharply. The weight of the intraperitoneal cysts in the B6D2F_1 mice decreased 4 months after infection. A similar phenomenon on the subcutaneous Echinococcus cysts was previously described by Desser (1963) in B6AF_1 mice.

The infected LD_1F_1 hybrids yielded an average of 7.31 gm. of the intraperitoneal cysts at 70 DPI. This value is significantly higher than 1.75 gm. in infected B6D2F_1 controls (Group I) and 2.29 gm. in splenectomized B6D2F_1 mice (Group II) at 71 DPI (Table VI).

The cysts of the splenectomized and the control mice did not differ in macroscopic appearance and in their distribution in the abdominal cavity. No protoscoleces were found in both groups of B6D2F_1 mice up to 131 DPI.

Responses of the host (Table VII) The rate of mortality both in infected controls (Group I) and splenectomized mice (Group II)

Figure 14

Mortality rate of splenectomized $B6D2F_1$ male mice infected with *Echinococcus multilocularis*

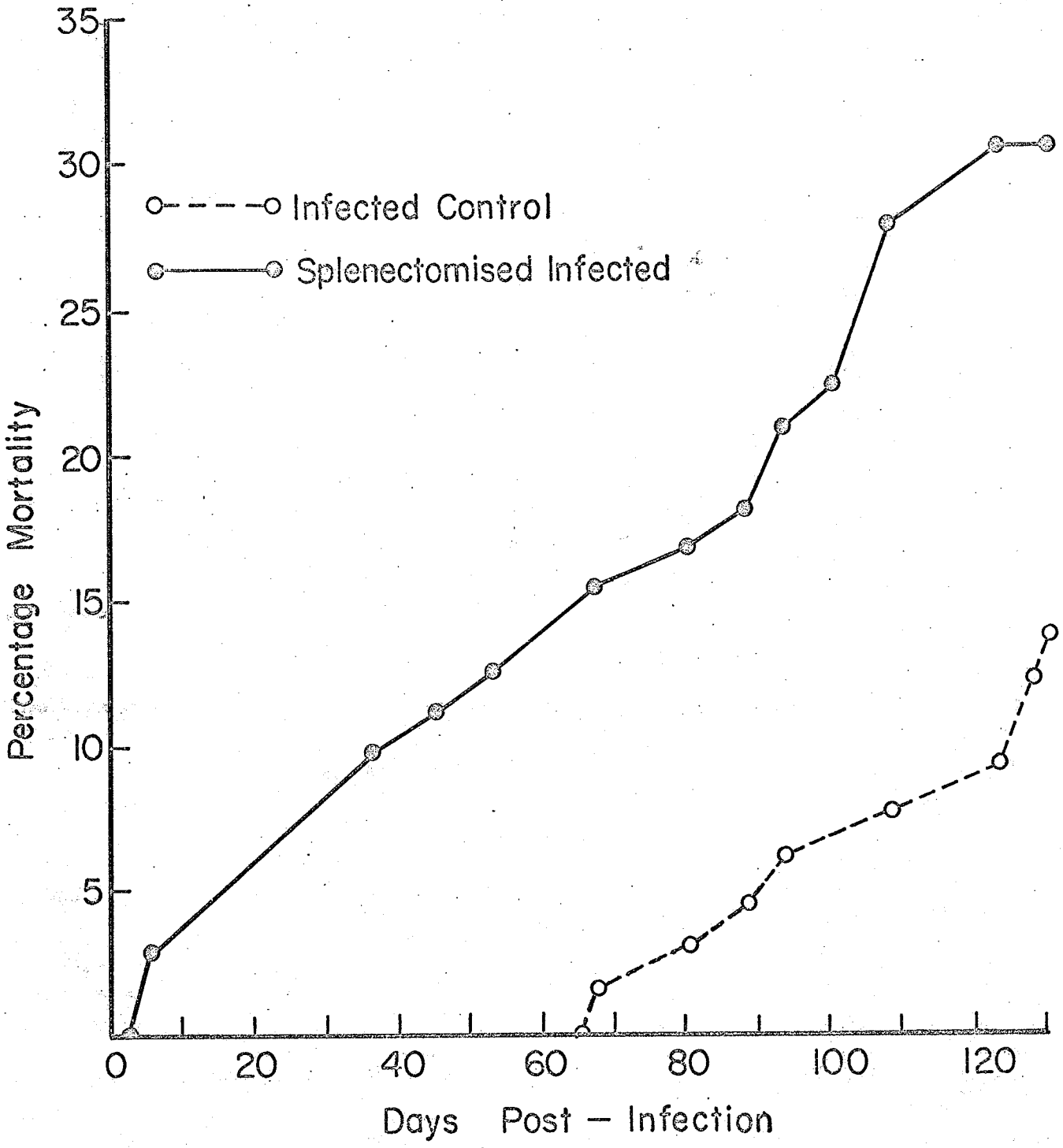

35
30
25
20
15
10
5
Percentage Mortality
Infected Control
Splenectomised Infected
0
20
40
60
80
100
120
Days Post – Infection

Figure 14

increased steadily with the age of infection (Fig. 14); however, the first death among the splenectomized infected hybrids occurred 6 days after infection, whereas, that in the infected controls 68 days after infection. At 131 DPI, Group II had a rate of 30.5% compared to 13.6% in the infected controls. No natural deaths were observed in uninfected, splenectomized mice (Group IV).

Splenectomy did not affect the body weight of $B6D2F_1$ hybrids (Fig. 15A). The mean "net" body weight, gross body weight minus the weight of Echinococcus cysts, remained more or less constant in Group I and Group II. The splenectomized uninfected controls (Group IV) had a lower mean body weight than the healthy uninfected controls (Group III) before 40 DPI, but soon Group IV overtook Group III and the body weight of both groups continued to increase.

The average weight of the spleen of a normal $B6D2F_1$ mouse is about 0.07 gm. Changes in the splenic weight of the infected $B6D2F_1$ controls (Group I) differ from those of infected LD_1F_1 hybrids in the second series of splenectomy experiments. In infected $B6D2F_1$ mice (Group I) the spleen weight increased to about 0.3 gm. 37 days after infection and remained constant up to 110 DPI (Fig. 15B). A further increase in splenic weight occurred 131 days after infection; the average splenic weight was 0.47 gm.

The weight of the kidney in splenectomized animals, both infected mice (Group II) and uninfected controls (Group IV), increased from the normal value of 0.20 gm. to about 0.26 gm. at about 110 DPI (Fig. 15C). Then the kidney weight decreased to 0.22 gm. and 0.25 gm. respectively.

Figure 15

Host responses of splenectomized B6D2F$_1$ male mice. A. "Net" body weight ---= gross body weight minus the weight of *Echinococcus* cysts; B. Spleen weight; C. Kidney weight; D. Absolute leucocyte count at autopsies.

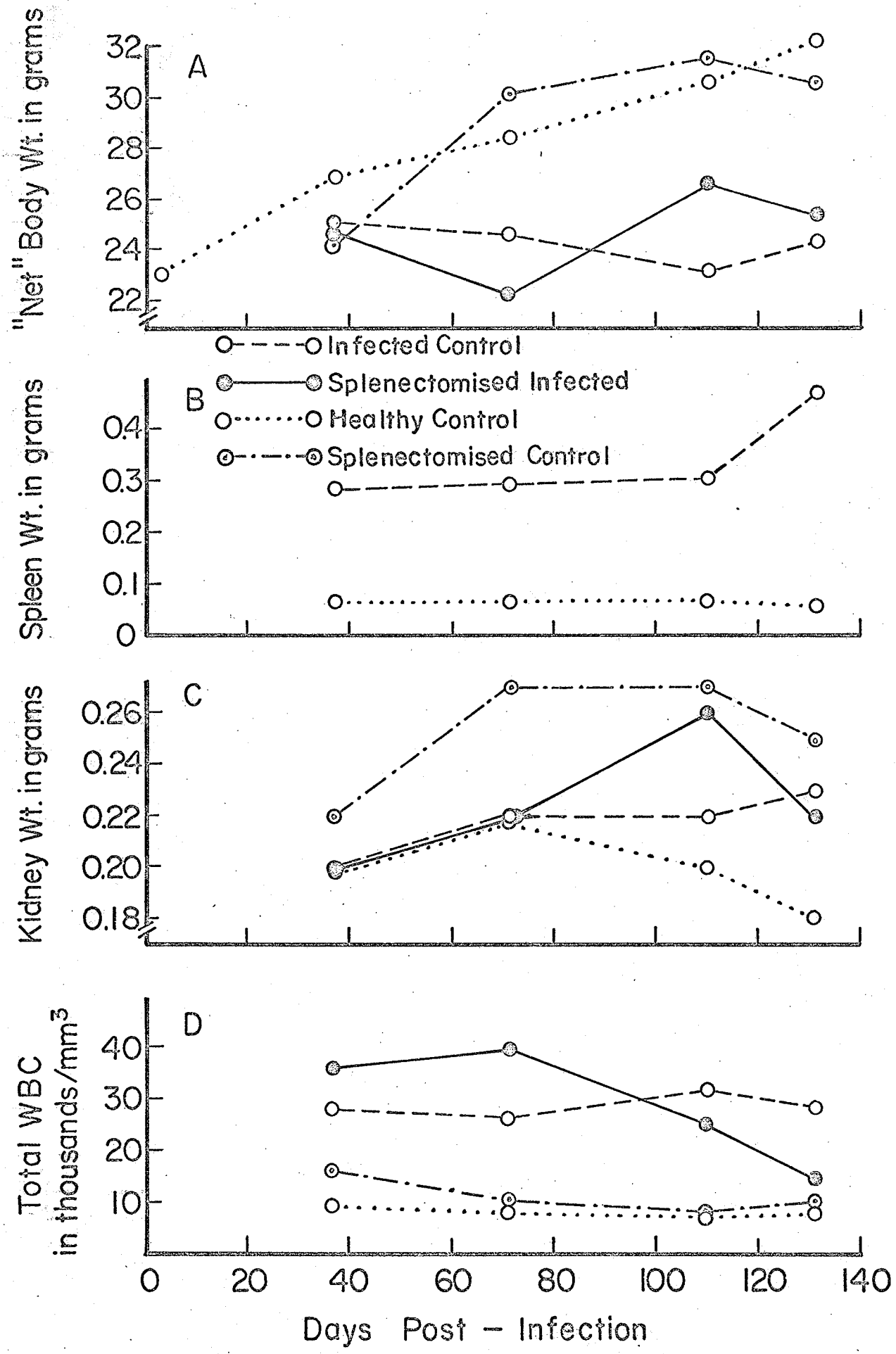

A
"Net" Body Wt. in grams
32
30
28
26
24
22
Infected Control
Splenectomised Infected
Healthy Control
Splenectomised Control
B
Spleen Wt. in grams
0.4
0.3
0.2
0.1
0
C
Kidney Wt. in grams
0.26
0.24
0.22
0.20
0.18
D
Total WBC in thousands/mm³
40
30
20
10
0
20
40
60
80
100
120
140
Days Post – Infection

Figure 15

Changes in the total leucocyte count in the blood are shown in Table VII and Fig. 15D. The normal $B6D2F_1$ males had an average total leucocyte count of about 8,400 cells per cu. mm. This is lower than 11,430 $\pm$ 1,040 cells/cu. mm. in C57Bl/6 mice and 9,280 $\pm$ 290 cells /cu. mm. in DBA/2 mice (Russel & Bernstein, 1966) C57Bl/6 and DBA/2 mice are the two parental strains of $B6D2F_1$ hybrid. In splenectomized uninfected mice (Group IV) splenectomy caused leucocytosis, and so did the implantation of Echinococcus cysts into the intact mice. Thirty-seven and 71 days after infection the splenectomized infected mice (Group II) had a higher total leucocyte count than the infected controls (Group I). Later the total leucocyte count in splenectomized, infected mice (Group II) decreased from the average of 39,460 cells/cu. mm. at 71 DPI to 14,700 cells/ cu. mm. 131 days after infection. At the same time, the total leucocyte count in Group I remained steady at about 30,000 cells/ cu. mm.

3. Cortisone and the growth of Echinococcus Cysts

The effect of cortisone acetate (Cortone, Merck and Dohme, Canada) on the growth of Echinococcus cysts in experimental mice was investigated. The experiments with cortone treatment were made in 3 stages:

a. drug tolerance test;
b. preliminary experiment with cortone (Experiment I)
c. protracted cortone treatment (Experiment II)

In all cases LD_1F_1 hybrid mice were used.

a. Drug Tolerance Test

Preliminary experiments made by Miss Margaret Puls indicated that cortone, given in a dose of 10 mg./kg., twice a week, slightly increased the growth of intraperitoneal Echinococcus cysts in C57L mice.

To test the tolerance of mice to cortone, four LD_1F_1 mice were given subcutaneous injections of cortone in doses of 500, 125, 50 and 25 mg./kg. at 3-day intervals. Five injections were made.

The mice which received 500 and 125 mg./kg. died 3 and 21 days after the first injection. Mice which were injected 50 and 25 mg./kg. five times survived indefinitely.

b. Preliminary experiment with Cortone (Experiment I)

Fifty-three LD_1F_1 female mice were infected intraperitoneally with 63 day old Echinococcus cysts from the 54th transfer of cotton rat strain. They were divided into 3 groups. One group acted as infected controls. The first cortone-treated group received 2 subcutaneous injections of cortone: an initial injection of 250 mg./kg. one day after infection and a second injection of 20 mg./kg. 7 days later. The mice of the second cortone-treated group received the same initial injections plus nine more doses of 20 mg./kg. each, twice a week. All mice were killed 40 days after infection. The results are summarized in Table VIII.

Table VIII

Growth of larval Echinococcus multilocularis in cortisone-treated LD_1F_1 mice

Group	Cortisone dosage mg./Kg.	Concentration index	Survival ratio	Autopsy DPI*	Gross wt. at autopsy gm.	Spleen wt. gm.	Cyst wt. gm.	t	p
Experiment I:- LD_1F_1 ♀♀ mice, 5 months old									
Control	-	0.33	16/16	40	28.3	0.38± 0.02	10.49± 0.41		
Treated #1	250x 1 plus 20x 1	0.33	19/19	40	32.6	0.34± 0.03	13.25± 0.57	3.95	< 0.001
Treated #2	250x 1 plus 20x10	0.33	15/18	40	27.4	0.24± 0.02	11.70± 0.41	2.08	< 0.05
Experiment II:- LD_1F_1 ♂♂ mice, $2\frac{1}{2}$ to $5\frac{1}{2}$ months old									
Control	-	0.19	12/-	24	28.9	0.11± 0.01	0.09± 0.04		
Treated	100x 1 plus 15x 5	0.19	15/-	24	26.6	0.09± 0.01	0.20± 0.04	1.91	> 0.05
Control	-	0.19	12/-	44	30.1	0.12± 0.01	0.25± 0.05		
Treated	100x 1 plus 15x 8	0.19	15/-	44	24.4	0.21± 0.02	3.36± 0.46	6.05	<0.001
Control	-	0.19	11/-	57	29.5	0.21± 0.04	1.36± 0.54		
Treated	100x 1 plus 15x11	0.19	14/-	57	22.6	0.16± 0.02	5.21± 0.44	5.60	<0.001
Control	-	0.19	10/-	70	30.9	0.43± 0.04	6.20± 1.02		
Treated	100x 1 plus 15x11	0.19	7/-	70	26.7	0.18± 0.02	8.05± 0.64	1.39	>0.10
Control	-	0.19	6/-	92	31.6	0.37± 0.04	10.46± 1.62		
Treated	100x 1 plus 15x11	0.19	1/-	92	22.2	0.07	8.08	-	-

* days post-infection

The autopsies of these mice showed the following results: (1) The growth of Echinococcus cysts was faster in both cortone-treated groups than in the controls, the difference in cyst weight being statistically significant at least at 0.05 level (Table VIII). (2) In the first cortone-treated group, the mean cyst weight was the highest, 13.25 gm. as compared to 10.49 gm. in the controls and 11.70 gm. in the second cortone-treated group. The mean spleen weight was 0.34 gm. compared to 0.38 gm. in the controls. The mice were not obviously emaciated. Note that the hormone was injected in the first week of infection. (3) The second cortone-treated group which received an initial dose of 250 mg./kg. plus ten subsequent doses of 20 mg./kg. had a lower cyst weight than the first cortone-treated group. Both the average body weight and the spleen weight of these mice were below those of the other two groups.

Thus initial doses of cortone given within 7 days after infection appear to facilitate the establishment of Echinococcus cysts. The highest dosage of cortone given to the second treated group seems to decrease the weight of cysts when compared with the first group. Again in this experiment, no protoscoleces were observed in the cysts.

c. Protracted cortone treatment (Experiment II)

The second experiment was planned to study the long term effects of cortisone treatment on the growth of E. multilocularis cysts (Table VIII). Male LD_1F_1 mice were chosen as experimental animals because the cysts grow more slowly in the males than in

the females. To slow down the growth of cysts further, a relatively high dilution of cyst material was used to infect the animals; the concentration index was 0.19. A total of 183 mice was used in this experiment. The mice were divided into 4 groups:

Group I - 63 infected controls
Group II - 80 infected, cortone-treated mice
Group III - 20 normal, uninfected controls
Group IV - 20 uninfected, cortone-treated controls

The mice of Group I and Group II (a total of 143) were infected intraperitoneally with Echinococcus cyst material from a cotton rat carrying the 57th transfer. The cysts were 42 days old. Each mouse of Group II and Group IV received an initial dose 100 mg./kg. of cortone one day after infection and subsequent doses of 15 mg./kg. at 5 day intervals. The hormone was injected subcutaneously. Post-infection treatment was stopped 53 days after infection due to high mortality of the mice.

Both the infected controls (Group I) and the infected cortone-treated mice (Group II) were autopsied at the following intervals: 24, 44, 57, 70, 92 days after infection. The mice were dissected in groups of about 12 controls and 15 treated mice. The high mortality among cortone treated mice unfortunately limited the number of mice autopsied to 7 and only one 70 and 92 days after infection respectively. It is obvious that the numerical data based on the autopsied at these two time intervals are not reliable.

The results of this experiment are summarized in Tables VIII and IX.

Table IX

Host reponses of cortisone-treated LD_1F_1 male mice

	DPI*	Group I Infected control	Group II Infected cortisone-treated	Group III Normal control	Group IV cortisone-treated
Total cortisone dose mg./Kg.	24	0	175	0	175
	44	0	220	0	220
	57	0	265	0	265
	70	0	265	0	265
	92	0	265	0	265
Gross body weight gm.	-3	28.7 (63)**	28.3 (80)	27.1 (20)	29.3 (20)
	24	28.9± 1.1 (12)	26.6± 0.6 (15)	26.9± 1.1 (4)	25.7± 0.7 (3)
	44	30.1± 0.3 (12)	24.4± 0.4 (15)	28.4± 0.7 (6)	25.3± 1.5 (3)
	57	29.5± 0.6 (11)	22.6± 0.8 (14)	28.1± 1.0 (6)	28.6± 3.0 (3)
	70	30.9± 0.7 (10)	26.7± 1.0 (7)	29.1± 0.6 (8)	28.4± 3.5 (2)
	92	31.6± 1.0 (6)	22.2 (1)	32.0± 0.5 (6)	29.3± 0.5 (2)
Spleen weight gm.	24	0.11± 0.01(12)	0.09± 0.01(15)	0.07	0.06± 0.00 (3)
	44	0.12± 0.01(12)	0.21± 0.02(15)	0.08	0.04± 0.02 (3)
	57	0.21± 0.04(11)	0.16± 0.02(14)	0.07	0.05± 0.01 (3)
	70	0.43± 0.04(10)	0.18± 0.02(7)	0.07	0.05± 0.00 (2)
	92	0.37± 0.04 (6)	0.07± (1)	0.07	0.07 (1)
Kidney weight gm.	24	0.26± 0.01(12)	0.26± 0.01(14)	0.20	0.26± 0.01 (3)
	44	0.26± 0.01(12)	0.22± 0.01(15)	0.20	0.24± 0.01 (3)
	57	0.27± 0.01(11)	0.19± 0.01(14)	0.20	0.29± 0.03 (3)
	70	0.22± 0.01(10)	0.17± 0.01 (7)	0.19	0.28± 0.04 (2)
	92	0.21± 0.01 (6)	0.14 (1)	0.20	0.28 (1)

* days post-infection

** number in bracket indicates the number of mice used.

Growth of the cysts:- The difference in the weight of cysts between cortisone-treated mice (Group II) and controls (Group I) is statistically significant at a 0.001 level 44 and 57 days after infection. This period falls into the third phase of fast growth of cysts in cortisone-treated mice.

As in the protracted experiment with splenectomized LD_1F_1 mice, the growth curves of the cysts were also sigmoid at least in the early phases (Fig. 16). In the infected controls (Group I), the initial phase of slow growth lasted about 40 days; the second phase of accelerating growth started 40 days after infection and lasted till about 55 DPI when the growth of the cysts became very fast. In the third phase of fast growth, the growth was directly proportional to time and expressed thus by an almost straight line on the graph. This phase continued to the end of the experiment.

In contrast to what occurred in infected controls, the first phase of slow growth in cortisone-treated mice (Group II) lasted only 20 days as compared to over 40 days in the controls. After a short period of acceleration, the stage of fast rectilinear growth followed, and continued to 70 days after infection. At that time only a few mice survived. The data about the only mouse in this group which survived 92 DPI was not plotted in Fig. 16.

The difference in the weight of cysts from cortisone-treated (Group II) and from control mice (Group I) was already obvious after 24 days of growth. This difference became highly significant at 44 days after infection (Table VIII). It is interesting that 57 days after infection the mean weight of the cysts from treated

Figure 16

Growth of larval Echinococcus multilocularis in cortisone treated LD_1F_1 male mice

A single initial dose of 100 mg./kg. and subsequent doses of 15 mg./kg. each were injected subcutaneously at 5-day intervals. Post-infection treatment was stopped after 53 DPI due to high mortality rate.

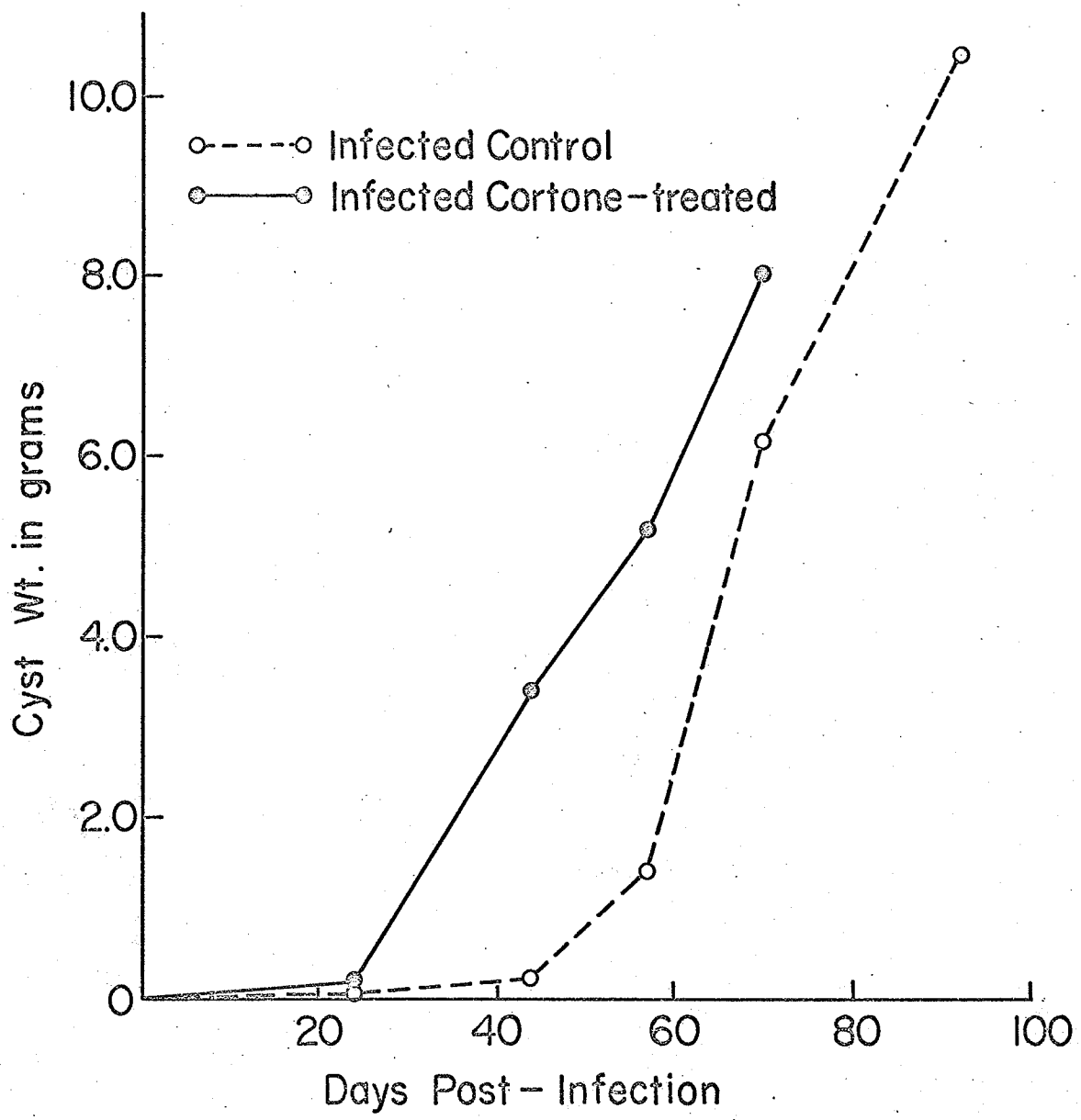

10.0
8.0
6.0
4.0
2.0
0
Cyst Wt. in grams
Infected Control
Infected Cortone-treated
20
40
60
80
100
Days Post-Infection

Figure 16

animals was 5.21 gm., and that from the controls only 1.36 gm. After 70 days of growth, the difference became much smaller: 6.20 gm. in the controls and 8.05 gm. in the cortone-treated mice. These last figures are, however, based on only ten controls and 7 treated animals.

It is interesting to note that in the period of fast growth the rate of cysts in controls seemed to be greater than in the cortisone-treated mice (Fig. 16).

In this experiment as in the previous ones, no protoscoleces were found in the cysts even 92 days after infection.

Responses of the host:- The observations on the responses of the hosts are summarized in Table IX. Before 44 DPI, there was no obvious difference in mortality between Group I and Group II (Fig. 17). Since then, the mortality in both groups increased considerably and was much faster in the treated mice (Group II) than in the controls. The cortisone treatment was stopped 53 days after infection due to this high mortality. Ninety-one days after infection Group II had an accumulated mortality of 32.5% compared to 19.1% in Group I. No death occurred in Groups III and IV.

The presence of Echinococcus cysts in the mice caused a decrease in net body weight, that is, gross body weight minus the weight of cysts (Fig. 18A). This decrease in body weight started at the time of the acceleration of the growth of the cysts, that is: 40 to 55 DPI in Group I and 20 to 30 DPI in Group II.

Figure 17

Accumulated mortality of cortisone treated LD_1F_1 male mice infected with Echinococcus multilocularis

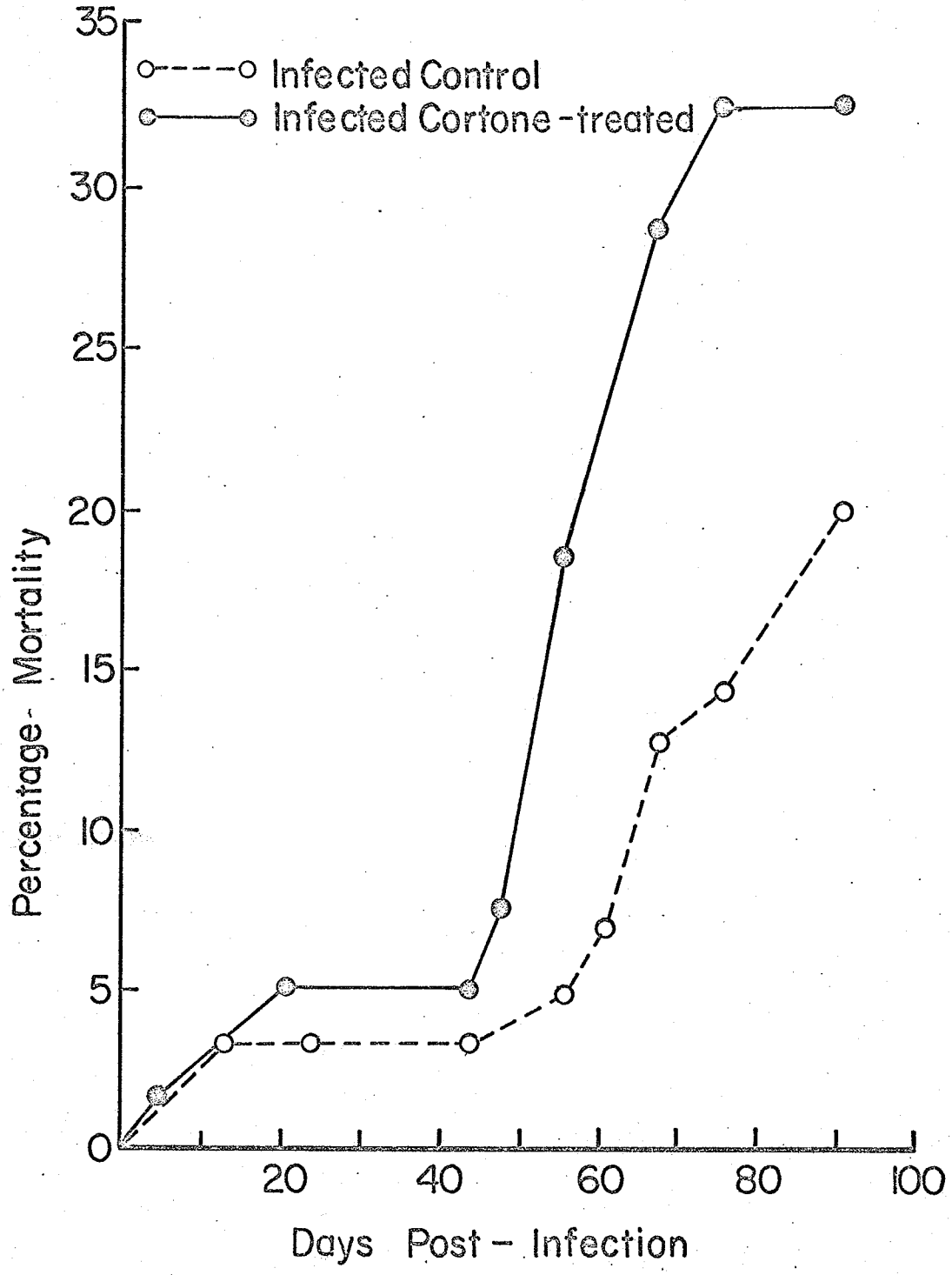

Infected Control
Infected Cortone-treated
Percentage- Mortality
Days Post – Infection
0
5
10
15
20
25
30
35
20
40
60
80
100

Figure 17

Figure 18

General host responses of cortisone treated LD_1F_1 male mice

A. "Net" body weight ----gross body weight minus weight of Echinococcus cysts; B. Spleen weight; C. Kidney weight.

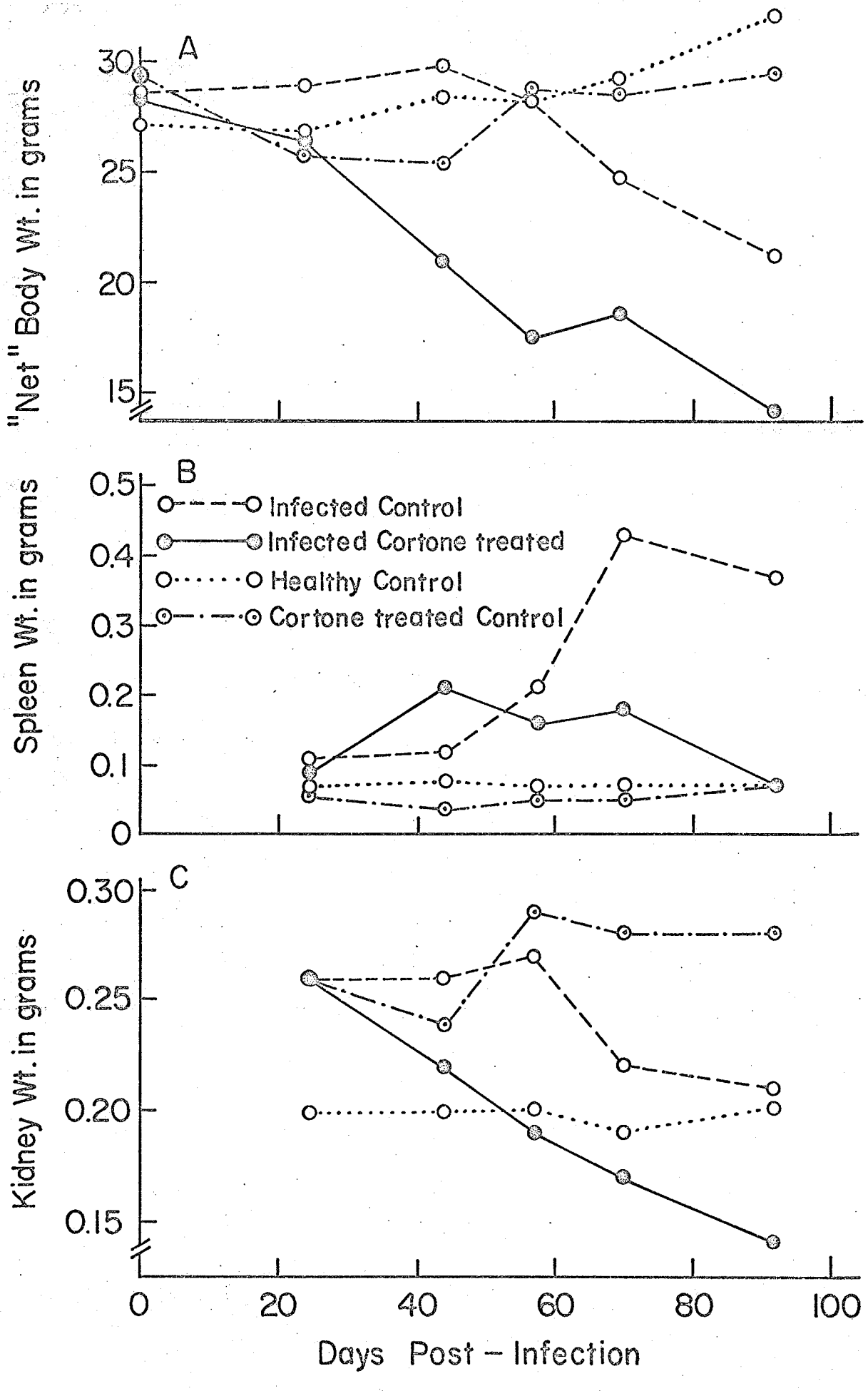

A
"Net" Body Wt. in grams
30
25
20
15
B
Spleen Wt. in grams
0.5
0.4
0.3
0.2
0.1
0
Infected Control
Infected Cortone treated
Healthy Control
Cortone treated Control
C
Kidney Wt. in grams
0.30
0.25
0.20
0.15
0
20
40
60
80
100
Days Post – Infection

Figure 18

The treated mice (Group II) lost net body weight faster than the infected controls (Group I). The weight of normal controls (Group III) increased steadily. In uninfected treated mice (Group IV), a loss of body weight was observed before 53 DPI. However, there was no obvious difference in body weight between Group III and Group IV mice after 53 DPI, when the cortisone treatment was stopped.

Cortisone caused a decrease in splenic weight in Group II and Group IV mice as compared to their controls, groups I and III (Figure 18B). The infected controls (Group I) developed a splenomegaly which reached a peak 71 days after infection and later subsided. These results are similar to those obtained in experiments with splenectomized male LD_1F_1 mice (Fig. 11). The maximum splenic weight in infected mice was always reached during the third phase of the fast, rectilinear growth of cysts: 57 DPI in Group II and 70 DPI in Group I (Fig. 16 18B).

Cortisone increased the average weight of the left kidney in uninfected LD_1F_1 hybrids (Fig. 18C). But in infected cortisone-treated mice (Group II) there was a considerable decrease in kidney weight from an average of 0.26 gm. 24 days after infection to 0.17 gm. 70 days after infection.

Changes in the blood count The absolute and the differential leucocyte counts in the blood are given in Tables X and XI. The absolute counts of different leucocytes are illustrated in Fig. 19.

Table X

Absolute leucocyte counts in cortisone-treated LD_1F_1 male mice

	DPI *	Group I infected control	Group II infected cortisone-treated	Group III healthy control	Group IV cortisone-treated control
Total WBC per mm^3	24	9,590± 920 (10)**	13,660± 1,600 (13)	7,920± 760 (4)	5,610± 2,520 (3)
	44	10,640± 970 (11)	39,190± 6,160 (12)	7,700± 1,180 (6)	4,190± 550 (3)
	57	18,660± 4,500 (11)	44,440± 9,330 (7)	5,310± 1,410 (6)	2,570± 1,100 (3)
	70	56,450±10,130 (10)	32,290± 7,610 (5)	9,950± 1,920 (8)	5,310± 1,340 (2)
	92	47,660± 9,000 (5)	-	9,130± 1,100 (5)	5,630± 2,270 (2)
Lymphocytes per mm^3	24	6,370± 670 (10)	3,350± 670 (12)	6,080± 890 (4)	3,360± 1,960 (3)
	44	5,140± 750 (11)	4,730± 670 (12)	4,940± 1,330 (6)	2,170± 690 (3)
	57	6,870± 1,080 (11)	5,860± 1,010 (7)	4,300± 1,290 (6)	1,600± 360 (3)
	70	11,950± 2,640 (10)	3,530± 700 (5)	7,670± 2,060 (8)	4,090± 760 (2)
	92	8,220± 1,590 (5)	-	7,100± 1,070 (5)	4,060± 1,720 (2)
Monocytes per mm^3	24	560± 140 (10)	1,300± 110 (12)	650± 130 (4)	530± 160 (3)
	44	720± 160 (11)	2,690± 530 (12)	470± 130 (6)	150± 60 (3)
	57	1,240± 260 (11)	1,790± 520 (7)	210± 80 (6)	80± 40 (3)
	70	6,300± 1,220 (10)	2,910± 810 (5)	490± 90 (8)	150± 30 (2)
	92	4,620± 1,030 (5)	-	800± 120 (5)	550± 70 (2)
Neutrophils per mm^3	24	2,610± 370 (10)	9,210± 1,690 (12)	1,180± 190 (4)	1,720± 330 (3)
	44	4,730± 710 (11)	31,700± 6,140 (12)	2,280± 490 (6)	1,850± 360 (3)
	57	10,490± 3,370 (11)	36,400± 8,200 (7)	750± 220 (6)	890± 730 (3)
	70	38,060± 6,860 (10)	25,770± 6,450 (5)	1,880± 540 (8)	1,070± 610 (2)
	92	34,730± 7,210 (5)	-	1,210± 240 (5)	1,060± 460 (2)
Eosinophils per mm^3	24	57± 27 (10)	270± 83 (12)	10± 10 (4)	5± 5 (3)
	44	60± 23 (11)	82± 29 (12)	15± 7 (6)	13± 7 (3)
	57	33± 15 (11)	102± 51 (7)	34± 17 (6)	0 (3)
	70	141± 114 (10)	85± 53 (5)	19± 10 (8)	0 (2)
	92	95± 95 (5)	-	25± 11 (5)	0 (2)

* days post-infection
** number in bracket indicates the number of mice used.

Table XI

Differential leucocyte counts in cortisone-treated LD_1F_1 male mice

	DPI *	Group I Infected control	Group II Infected cortisone-treated	Group III Healthy control	Group IV Cortisone-treated control
% lymphocytes	24	66.2± 2.6 (12)**	26.9± 5.4 (12)	76.0± 4.3 (4)	57.8± 4.4 (3)
	44	48.9± 5.1 (11)	15.3± 3.1 (12)	62.3± 5.7 (6)	50.9± 3.5 (3)
	57	46.3± 6.4 (11)	14.4± 1.5 (7)	79.4± 4.9 (6)	66.0± 8.3 (3)
	70	23.3± 3.0 (10)	13.9± 3.8 (5)	73.2± 5.3 (8)	77.7± 3.7 (2)
	92	17.0± 1.7 (5)	-	77.3± 3.2 (5)	71.3± 1.3 (2)
% monocytes	24	4.9± 0.9 (12)	9.8± 0.9 (12)	8.8± 1.9 (4)	9.2± 1.7 (3)
	44	6.4± 0.9 (11)	6.3± 0.8 (12)	6.7± 2.0 (6)	4.1± 2.0 (3)
	57	6.6± 1.2 (11)	5.5± 0.8 (7)	3.9± 0.9 (6)	2.8± 0.9 (3)
	70	11.1± 1.0 (10)	8.1± 1.9 (5)	4.8± 0.6 (8)	3.[illegible]± 1.0 (2)
	92	9.8± 1.8 (5)	-	9.2± 1.4 (5)	10.3± 1.8 (2)
% neutrophils	24	27.8± 2.9 (12)	61.4± 5.6 (12)	15.3± 2.6 (4)	33.0± 3.4 (3)
	44	44.3± 4.6 (11)	78.2± 3.2 (12)	30.8± 4.5 (6)	44.5± 1.8 (3)
	57	47.0± 5.7 (11)	80.6± 1.9 (7)	15.4± 4.2 (6)	31.2± 7.5 (3)
	70	65.5± 3.2 (10)	77.8± 2.4 (5)	22.4± 5.4 (8)	19.4± 4.7 (2)
	92	73.0± 3.2 (5)	-	13.2± 2.0 (5)	18.5± 0.5 (2)
% eosinophils	24	0.7± 0.4 (12)	1.7± 0.4 (12)	0.1± 0.1 (4)	0.1± 0.1 (3)
	44	0.6± 0.3 (11)	0.3± 0.1 (12)	0.2± 0.1 (6)	0.3± 0.2 (3)
	57	0.2± 0.1 (11)	0.2± 0.1 (7)	0.5± 0.2 (6)	0 (3)
	70	0.2± 0.1 (10)	0.2± 0.1 (5)	0.2± 0.1 (8)	0 (2)
	92	0.2± 0.2 (5)	-	0.3± 0.1 (5)	0 (2)

* days post-infection

** number inbracket indicates the number of mice used.

The normal male LD_1F_1 mice had a total leucocyte count of about 8,000 cells /mm^3. with 19.4 percent neutrophils. These figures are close to those reported by Russel and Bernstein (1966): 8,600± 1,600 leucocytes /mm^3. with 20.4± 4.2 percent granulocytes in DBA/1 males, one of the parental strains of LD_1F_1 hybrids.

No basophils were seen in the blood of LD_1F_1 mice. The scarcity of these cells in the blood of the mice is well known (Bloom and Fawcett, 1964; Russell and Bernstein, 1966; Reed and Jutila, 1967).

In my experiments, Echinococcus infection caused a pronounced leucocytosis and spleen enlargement. The changes in total leucocyte count followed those of the splenic weight (Fig. 18B). The maximal count, 56,450 cells/mm^3. in infected controls (Group I) coincided with the peak of the splenic weight of 0.43 gms. at 70 DPI. The same was true for infected, cortisone-treated mice (Group II) 57 days after infection when the peak leucocyte count was 44,440 cells/mm^3.

Analysing the changes in the absolute and differential leucocyte counts in the course of echinococcosis in LD_1F_1 mice (see Fig. 19) one can come to the following conclusions:

(1) In early stages of infection the total leucocyte count roughly depends on the weight of the cysts. Cortisone tends to suppress the leucocytosis (Fig. 19A and Fig. 16).

(2) Cortisone caused lymphopenia, monocytopenia and eosinopenia, but had no effect on absolute neutrophil counts.

(3) In mice with Echinococcus cysts, the leucocytosis depends basically on neutrophilia, at least in the early stages of infection.

Figure 19

Leucocyte counts in the blood of cortisone treated LD_1F_1 male mice infected with Echinococcus multilocularis

A. total leucocyte count; B. absolute lymphocyte count; C. absolute neutrophil count; D. absolute monocyte count; E. absolute eosinophil count.

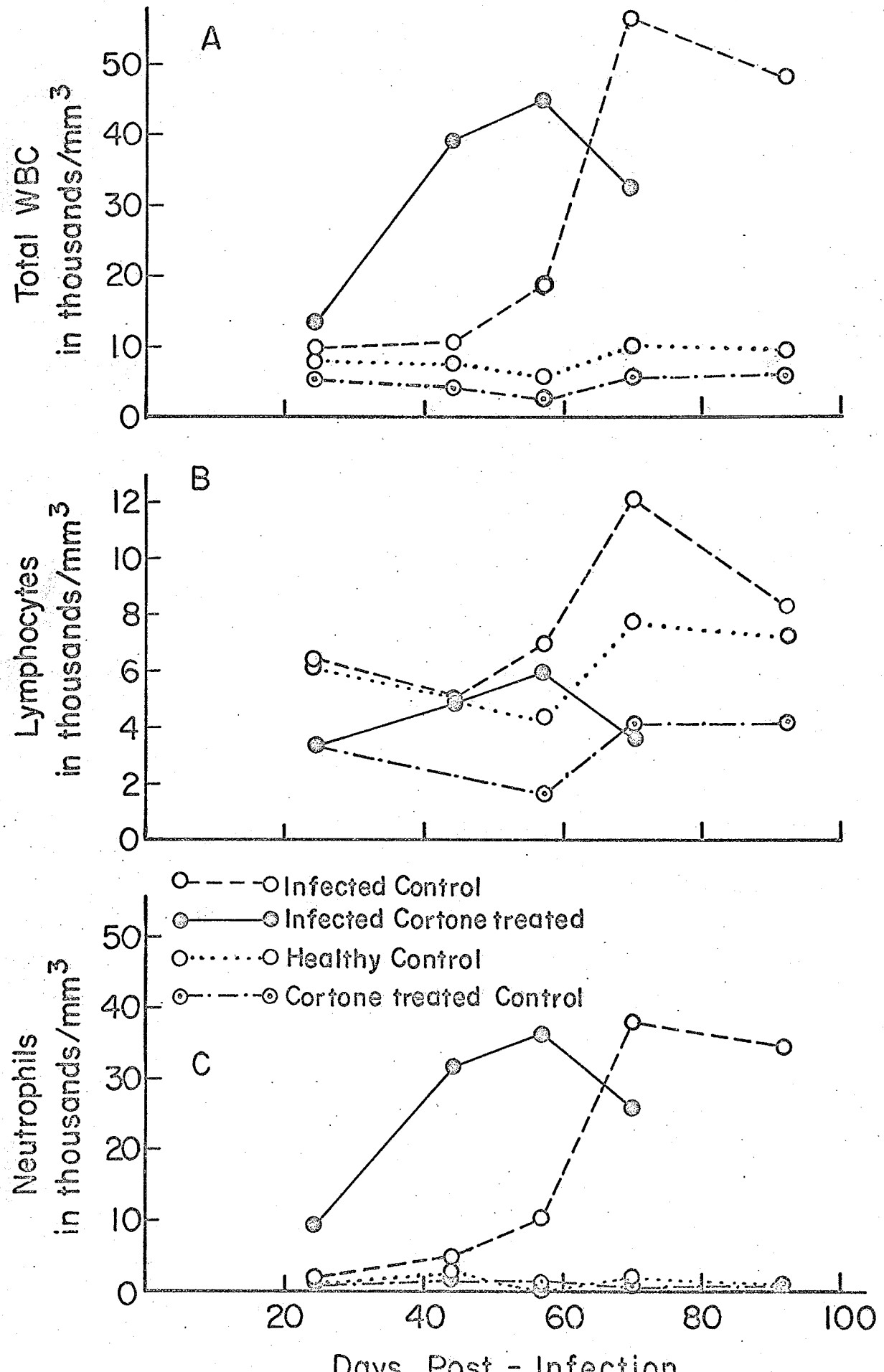

A
Total WBC
in thousands/mm3
0
10
20
30
40
50
B
Lymphocytes
in thousands/mm3
0
2
4
6
8
10
12
Infected Control
Infected Cortone treated
Healthy Control
Cortone treated Control
C
Neutrophils
in thousands/mm3
0
10
20
30
40
50
20
40
60
80
100
Days Post - Infection

Figure 19

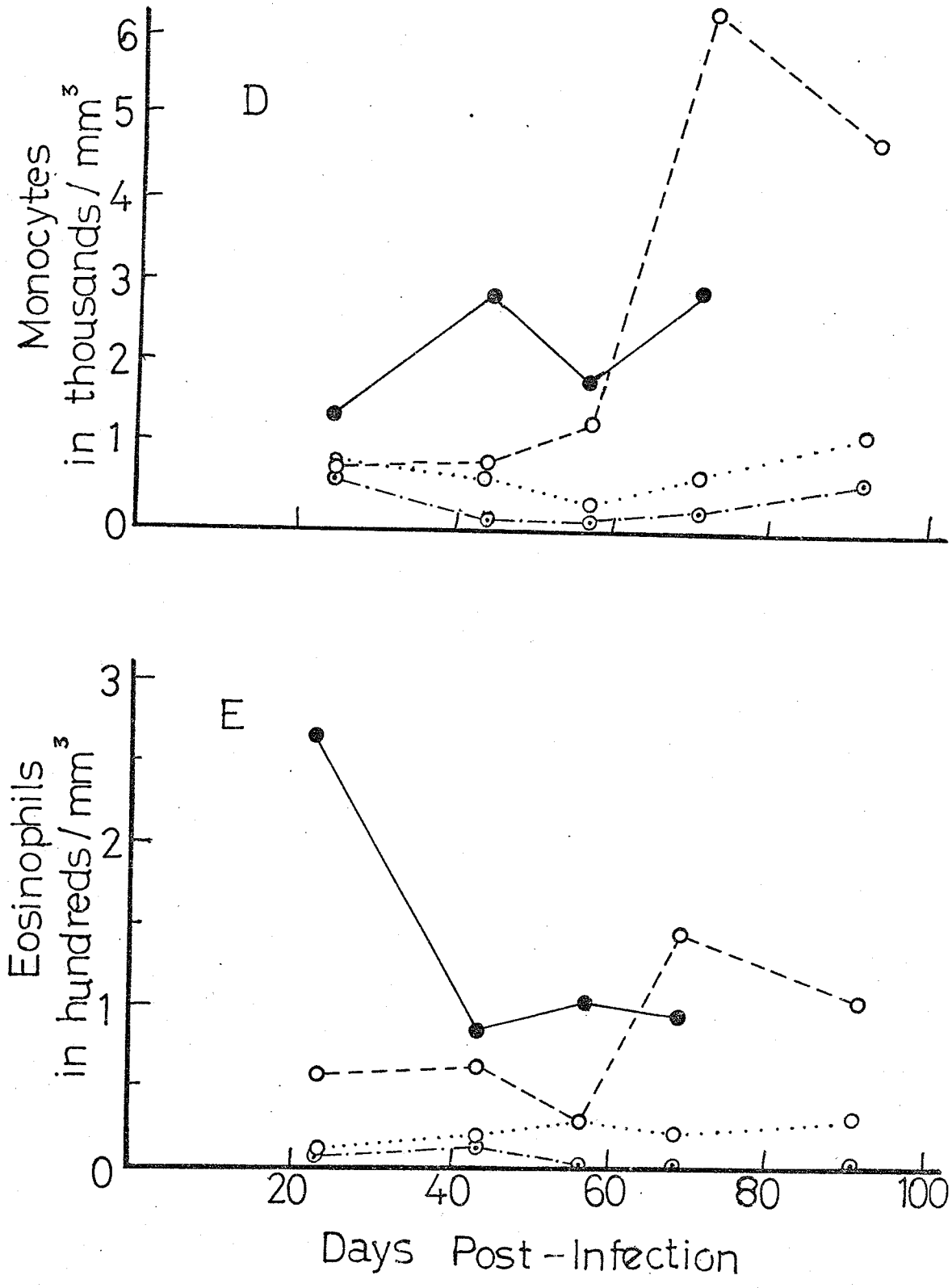

D
Monocytes
in thousands / mm³
0
1
2
3
4
5
6
E
Eosinophils
in hundreds / mm³
0
1
2
3
20
40
60
80
100
Days Post-Infection

Figure 19

Seventy days after infection, the total leucocyte count in infected controls (Group I) was 5.6 times higher than in the normal controls (Group III) whereas the neutrophil count in the infected controls was 20 times higher than that in the non-infected mice.

(4) Lymphopenia is responsible for the lower total leucocyte count in infected cortisone treated mice. For example, 57 days after infection, the total leucocyte count in the infected, cortisone-treated (Group II) mice was 8.4 times higher than that in the uninfected controls (Group III). But the lymphocyte count in the infected, cortisone-treated mice was only 1.4 times higher than that in the normal controls. The lymphocyte count is practically unaffected by the presence of Echinococcus cysts (Fig. 19B).

(5) Eosinophilia was not apparent in infected LD_1F_1 mice. The absolute eosinophil count never exceeded 200 cells/mm^3. and the percentage of eosinophils never exceeded 2. The absence of eosinophilia in mice infected with Echinococcus agrees with the data of Webster and Cameron (1961).

Obviously, the changes in total leucocyte counts do not run strictly parallel to the growth curves of the cysts in chronic Echinococcus infection. The total leucocyte count in infected mice decreased after they reached their maximum whereas the cysts were still growing (Fig. 16 and Fig. 19A).

DISCUSSION

1. Some sources of experimental errors

a. Cyst weight

Obviously, the clusters of cysts contain some host tissues. It is understood that the increase in the weight of cysts is not a very accurate measure of their growth. Still this criterion was accepted because of the absence of a better one.

The variability of the cyst growth in individual mice of the same strain and sex is great as indicated by the high probable error. To compensate for this variability relatively large numbers of mice were used in each group.

b. Mortality rate

The mortality is a cumulative death toll of mice expressed in percent. As the toll of mice autopsied earlier is not taken into consideration in subsequent enumerations, the actual mortality is higher than that recorded.

2. Evaluation of the experimental results

My experiments showed distinctly that neither the ink blockade nor the splenectomy has any significant effect on the growth of intraperitoneal Echinococcus cysts in the rodents studied. Cortisone accelerates the growth of Echinococcus cysts by shortening the first phase of slow growth. This effect is observable even when cortisone is given only in the course of the first week after infection.

The true nature of the defense mechanism of the host to Echinococcus infection remains uncertain.

a. Indian ink blockade of the RES

My experiments with the carbon particle blockade of the RES gave consistently negative results.

To arrive at a decisive conclusion, the effects of the mode of blockade and of the strain differences of the host were studied. As the cyst material was injected intraperitoneally, it seemed that a local, intraperitoneal blockade will be more efficient than a systemic one (by intravenous route). Both the CBA mice in which the cysts grow slowly and LD_1F_1 mice in which the cysts grow very fast were used. The results showed that these methods did not modify the growth of Echinococcus cysts.

It is known that the blockade of the RES changes the course of many protozoan infections, especially those with avian plasmodia (see review by Goble and Singer, 1960; Garnham, 1963). This probably depends on the impairment of phagocytosis by the RES. Morseth and Soulsby (1969) showed that neutrophils, in the presence of opsonic factor, dissolve and phagocytose the cuticle of Ascaris suum larvae in vitro. The inhibitory effect of the blockade of the RES on phagocytosis of carbon particles lasts for at least 3 days (Benacerraf, et al 1954). Therefore the results of my experiments seemed to indicate that the growth of intraperitoneal Echinococcus cysts is not inhibited by non-specific suppression of the phagocytosis by the macrophages.

It is thus probable that a temporary stimulation of RES into a hyperphagocytic state by such stimulants as zymosan and restim (See Machado, 1968; Heller, 1960) will not inhibit the growth of E. multilocularis cysts. It is also probable that an increase in the number of essential phagocytes in the blood will not inhibit the growth of these cysts. The above views are supported by the presence of a very high neutrophilia and monocytosis in infected LD_1F_1 mice in which Echinococcus cysts grow extremely fast (Fig. 19C and D).

The mice which received larger doses of ink had a higher mortality. This was presumably due to RES exhaustion. It is interesting to note that 70 days after infection the three , out of ten infected LD_1F_1 males which survived a heavy dose of 2000 mg. of carbon (commercial Pelikan ink) per kg. of body weight, had no cysts at all (Table II). It is possible that the cysts inoculated into these mice were killed or damaged by some ingredients of this Indian ink. Commercial Pelikan ink contains shellac as a stabilizing agent and adds lustre to the ink. Shellac is a resin consisting of polyhydroxy organic acids (predominantly aleuritic acid, $C_{16}H_{32}O_5$ and shellolic acid, $C_{15}H_{20}O_6$).

Finally, it would be interesting to compare my results with similar Indian ink blockade experiments of other authors. But no such data are available. It is difficult to assume that Taliaferro's (1940) suggestion to use Indian ink blockade in helminthic infections was not followed. Probably the absence of such data in the literature depends simply on the fact that the results of such experiments were negative and not published.

b. Splenectomy

Splenectomy does not change significantly the course of many helminthic infections in mammals. This is borne out by experiments with Schistosoma mansoni (Thompson, 1954; Bruce et al, 1966), Taenia taeniaeformis (Hoeppli, 1941), Hymenolepis nana (Larsh, 1944; Weinmann, 1968), Trichinella spiralis (Bittner, 1913), Nippostrongylus brasiliensis (Ogilvie & Jones, 1967), Aspicularis tetraptera (Mathies 1962), Dirofilaria immitis (Hawking, 1962), Loa loa (Duke, 1960 after Nooruddin and Ahmed, 1967), Litomosoides carinii (Hawking, 1962), Brugia malayi and B. pahangi (Ahmed, 1965 after Nooruddin and Ahmed, 1967).

It was therefore not surprising to find that splenectomy in mice did not affect the course of E. multilocularis infections in this project.

My experiments clearly establish three facts: (1) the spleen does not play an essential role in the natural susceptibility or resistance to E. multilocularis in mice, rats and gerbils; (2) the negative results obtained do not depend on the sex of the hosts; (3) the growth curves of cysts in splenectomized and control mice almost coincide both when LD_1F_1 mice and $B6D2F_1$ mice were used. Yet the growth curves of cysts in these two types of mice are different (Fig. 9 and 13).

Three interesting observations on the possible effects of splenectomy on the growth of Echinococcus cysts in mice were made:

Observation 1. In the male LD_1F_1 mice, the growth curves of cysts in the splenectomized and the control mice almost coincided in the course of the first 54 days of infection (Figure 9; Table IV).

From that time on, these curves diverged. Sixty-two days after infection, the mean weight of cysts in the 11 splenectomized animals was 10.05 ± 1.26 gm. and in the 10 controls 6.59 ± 1.76 gm. The difference in weight, though large, is not statistically significant. In the second experiment with LD_1F_1 females, the mean cyst weight of mice dissected at 77 DPI was 5.73 ± 1.21 gm. for the 24 splenectomized and 4.07 ± 1.14 gm. in the 12 controls. The difference is also not significant. The fact that in both experiments with LD_1F_1 mice, cysts were heavier in the splenectomized animals than in the controls suggests that the difference is not accidental.

Observation 2 In the late phases of infection in $B6D2F_1$ mice, the weight of Echinococcus cysts was less in splenectomized than in control mice (Fig. 13). The difference was not statistically significant. Interestingly the cyst weight in $B6D2F_1$ mice was less than in the controls whereas in LD_1F_1 mice the splenectomized animals had larger cysts than the control. Whether the differences in cyst weight at the end of the experiment are accidental or not needs further verification.

Observation 3 The total leucocyte count in the blood is not a good indicator of the resistance of the host to Echinococcus infections (Table V and VII; Fig. 15).

The observations 1 and 2 on the growth of cysts in the LD_1F_1 and $B6D2F_1$ mice are difficult to reconcile. Bruce et al (1966) found that in splenectomized monkeys Schistosoma mansoni passed more eggs for a longer period than the controls. Elks and Cantrell (1966) found that the phagocytosis in splenectomized rats infected

with Plasmodium berghei did not differ from that in controls in the course of the first 7 days after infection, but later became less efficient than that in the controls. These results combined with my first observation (that the splenectomized LD_1F_1 mice had, at the end of the experiment (Fig. 9), more Echinococcus cysts than the controls) suggests the following possibility. The spleen is not necessary for the development of immunity, but may still be important for the maintenance of the enhanced RE activity in the late stages of infections in LD_1F_1 mice.

My second observation on splenectomized infected $B6D2F_1$ mice (Fig. 13) seems to indicate the development of a delayed immunity to Echinococcus in these resistant hosts. Serological studies on $B6D2F_1$ mice in late stages of Echinococcus infection, 100 days after infection, would be interesting. Such studies may show the difference in antibody titres between splenectomized and intact mice. It is known that splenectomized animals respond to a continual antigenic stimulation with a delayed and yet higher antibody titre than the controls (see Taliaferro, 1956).

The third observations on the leucocyte counts suggests that the resistance of mice to Echinococcus infection is not proportional to the total number of leucocytes. Freeman (1964) made similar observations in mice infected with Taenia crassiceps. In $B6D2F_1$ mice with intraperitoneal E. multilocularis cysts, there was a late increase in splenic weight, but no corresponding increase in the total leucocyte count (Fig. 15B and 15D). Whether this spleen enlargement was associated with an increase in antibody production remains to be investigated. Studies on the lymphocyte

counts in $B6D2F_1$ mice may contribute to the solution of this problem.

In summary, my experiments indicate that splenectomy does not increase the growth rate of E. multilocularis cysts in the rodents examined.

c. Cortisone and the growth of Echinococcus multilocularis cysts

My experiments showed that cortisone has a profound effect on the growth of larval E. multilocularis in the abdominal cavity of LD_1F_1 mice. The major observations are:

(1) Cortisone was effective when given in the first 7 days after infection (Experiment 1, Table VIII).

(2) Cortisone shortened the first phase of slow growth of the cysts (Fig. 16). This phase lasted 20 days in the cortisone-treated mice as compared to 40 days in the controls. In the cortisone-treated mice, the weight of the cysts was higher than in the controls; but the difference was statistically significant only 44 and 57 days after infection.

(3) Cortisone did not promote the development of protoscoleces; they were absent even in the 92 day-old cysts. (In LD_1F_1 mice the cysts became fertile about 3 to 4 months after intraperitoneal infection with E. multilocularis cysts, and very few of these mice survive that long.)

Many authors found that cortisone impairs the resistance of mammalian hosts to infections with helminths belonging to different classes and even phyla, as already pointed out in the

in the review of the literature. Tada (1967) described the effects of cortisone in trematode infections, namely in those with Paragonimus miyazakii. Similar phenomena were observed in infections with cestodes, especially larval ones. In this respect, important are the papers of Weinmann et al (1961) on Hymenolepis nana, of Oliver (1962) on Hydatigera taeniaeformis and of Esch (1967) on Taenia multiceps. Research on the suppression of immunity to nematodes by cortisone was started well before the publication of the first paper on the cestode infections. Some of the more important papers on the nematodes and cortisone are those of Weinstein (1955) on Nippostrongylus muris, of Coker (1956) on Trichiaella spiralis, of Parker (1961) on Nippostrongylus brasiliensis, of Cross (1962) on Nematospiroides dubius, of Mathies (1962) on Aspicularis tetraptera, of Briggs (1963) on Litomosoides carinii, of Campbell (1963) on Trichuris muris and of Miller (1966) on Necator americanus.

Cortisone acts basically on the host resistance to certain stages in the life cycle of the helminths, especially on those which are in contact with host tissues. These reactions depend on many factors. Some of them are: mechanical or chemical injury to the tissued caused by the helminths, the antigenicity of the helminths and their metabolites, etc. Examples of the effects of cortisone on helminthic infections are the suppression of the encapsulation of Trichinella spiralis in hamsters (Ritterson, 1959) and the inhibition of immune responses to hexacanths of Hydatigera taeniaeformis in mice (Oliver, 1962). Obviously, cortisone basically affects the the responses of the hosts and not those of the helminths. However, the direct action of cortisone on the helminths cannot be completely excluded.

Oliver (1962) showed that cortisone when given 4 to 12 days after infection facilitates greatly the establishment of the hexacanths of Hydatigera taeniaeformis in mice. He explained this fact by referring to the observations of Miller and Gardiner (1934) that in rats the antibody production starts about 7 days after infection, and that the titres become high a week later. Oliver also suggested that the tissue reactions against H. taeniaeformis larvae develop predominantly in the cource of the second week after infection, thus simultaneously with the onset of antibody production. Similar results were obtained by Esch (1967) who worked with Taenia multiceps in mice. He showed that cortisone was most efficient when injected 1 to 14 days after infection. It is known also that the hexacanths of another taeniid, Echinococcus granulosus, evoke a strong antigenic response in sheep, which develop an "early immune response" (Gemmell, 1962 and 1966). Mankau (1957) infected white mice with onchospheres of E. multilocularis and found that the inflammatory reactions in in the liver started 6 days after the infection and persisted for over 3 months.

In my experiments with E. multilocularis in LD_1F_1 mice, intraperitoneal infection with cyst material was used. Obviously, the stage of hexacanth was missing and the cysts developed basically not in the liver but in the abdominal cavity, predominantly on the mesenteries and the omenta. My results, therefore, are not directly comparable to those in which onchospheres were used to infect animals. Whether the shortening of the initial phase of slow growth of

Echinococcus cysts in cortisone treated LD_1F_1 mice (Fig. 16) depends on the inhibition of the "early immune response" or the suppression of tissue reactions, cannot be decided on the basis of my material. Both serological and histopathological studies are needed to evaluate the roles of the humoral and cellular factors in the defense of the host. It is emphasized that the serological and the histological factors are closely interrelated and represent merely two different aspects of an integrated host reaction.

It may be interesting to discuss two probable effects of cortisone on the growth of E. multilocularis cysts.

(1) In my first experiment with cortisone in which very large doses were used: in one treated group, an initial dose of 250 mg./kg. plus only one subsequent dose of 20 mg./kg. and in the other treated group the same large initial dose plus ten doses of 20 mg./kg. at 3-day intervals. The mean cyst weight was smaller in mice which got ten subsequent injections than in those which received only one subsequent injection (Experiment 1, Table VIII). The mean weight of cysts was 11.70± 0.41 gm. in the group which received more cortisone, and 13.25± 0.57 gm. in the group which received less. The difference in cyst weight was 1.55 gm. and the t value 2.2.

(2) In the second experiment with cortisone, in which the phase of slow growth in treated mice was much shorter than in the controls, the slope of the growth curve was steeper in control mice than in the cortisone-treated (Fig. 16).

These two observations indicate that cortisone under some circumstances may retard the growth of Echinococcus cysts. Two explanations of this phenomenon are possible: (1) direct suppressive action of cortisone on the cyst and (2) the detrimental effects of cortisone on the host, resulting in a poor condition for the cysts. However, the differences in the weight of cyst on which these explanations are based are not statistically significant.

Finally it is interesting to point out that the lymphocyte count in the LD_1F_1 mice is practically not affected by the presence of Echinococcus cysts (Fig. 19B). Whether this fact suggests an immune incompetence of LD_1F_1 mice in echinococcosis or not needs further research.

CONCLUSIONS

1. The blockade of the reticuloendothelial system does not affect the growth of larval *Echinococcus multilocularis* in LD_1F_1 and CBA mice.

2. Splenectomy does not significantly affect the growth of intraperitoneal *E. multilocularis* cysts in mice, rats, and gerbils.

3. Cortisone accelerates the growth of cysts in LD_1F_1 mice. This acceleration is the result of the shortening of the phase of slow growth.

4. The impairment of host resistance to *E. multilocularis* by cortisone occurs in the early period of growth of cysts. Further experiments are needed to determine the relative importance of tissue reactions and serological factors of this impairment.

SUMMARY

1. The effects of the blockade of the RES, splenectomy and cortisone on the growth of intraperitoneal Echinococcus multilocularis cysts in rodents were studied. The rodents used were LD_1F_1, CBA and $B6D2F_1$ mice, hooded rats and gerbils. Most experiments were made with LD_1F_1 mice in which the cysts grow extremely fast. Fertile cysts from the 46th to 57th transfers in cotton rats were used as a source of infection.

2. Carbon particle blockade did not affect the growth of intraperitoneal Echinococcus cysts in LD_1F_1 and CBA mice. The results were negative independent of the route of administration of the Indian ink (intraperitoneal or intravenous).

3. Preliminary experiments showed that splenectomy did not affect the growth of Echinococcus cysts in LD_1F_1 mice, gerbils and hooded rats.

4. Splenectomy experiments in which 185 LD_1F_1 and 186 $B6D2F_1$ mice were used confirmed the results of the preliminary experiments. The growth curve of cysts in the splenectomized and the control mice were almost identical.

5. Cortisone accelerated the growth of Echinococcus cysts in LD_1F_1 mice of both sexes. In a preliminary experiment, an initial large dose, 250 mg./kg. given one day after infection, and one subsequent dose, 20 mg./kg. given 6 days later, significantly increased the weight of cysts examined 40 days after infection.

6. In a protracted experiment, an initial dose of cortisone, 100 mg./kg., given one day after infection plus subsequent doses, 15 mg./kg., given twice a week, considerably accelerated the growth of Echinococcus cysts in LD_1F_1 mice. The period of the first phase of growth (slow growth) was much shorter in the cortisone-treated mice than in the controls, 24 and 40 days respectively. The greatest difference in the cyst weight between the treated and control mice was observed 44 to 57 days after infection.

7. In the early stages of Echinococcus infections, the mice developed a very high leucocytosis with pronounced neutrophilia but without lymphocytosis. Cortisone lowered the number of lymphocytes and monocytes in the blood, but had no effect on the absolute neutrophil count.

8. In LD_1F_1 mice, the growth curve of cysts is sigmoid but without the plateau. Most mice die in the rectilinear phase of growth.

9. In $B6D2F_1$ mice, the growth of cysts is much slower but of a similar pattern of growth as that in LD_1F_1 mice. However, the period of fast growth is followed by a slow decline in the mean cyst weight.

REFERENCE

Abramoff, P., Hintzke,C. and Brien,N. 1968. Effects of actinomycin-D on antibody synthesis by spleen cells of immunized rat. J. Reticulo-endothelial Society 5:498-509.

Agarwal, M.K. and Berry, L.J. 1968. Effects of actinomycin-D on the RES and development of tolerance to endotoxin in mice. J. Reticuloendothelial Society 5:353-367.

Agosin, M. and Repetto, Y. 1967. Studies on the metaboliam of Echinococcus granulosus. IX. Protein synthesis. Exp. Parasit. 21: 195-208.

Aschoff, L. 1924. Das reticulo-endotheliale System. Ergebn. inn. Med. u. Kinderh. 26: 1. (After Carpenter, 1965.)

Bailliff, R.N. 1960. Reaction patterns of the RES under stimulation. Ann. N.Y. Acad. Sci. 88: 3-13.

Barabash-Nikiforov, I. 1938. Mammals of the Commander Islands and the surrounding sea. J. Mamm. 19: 423-429.

Benacerraf, B. Halpern, B.N. Biozzi, G. and Benos, S.A. 1954. Quantitative studies of the granulopoietic activity of the reticuloendothelial system. IV. The effects of cortisone and nitrogen mustard on the regenerative capacity of the RES with saturation with carbon Brit. J. Exp. Path. 35: 97-106.

Benacerraf, B. and Miescher, P. 1960. Bacterial phagocytosis by the RES in vivo under different immune conditions. Ann. N.Y. Acad. Sci. 88: 184-195.

Benacerraf, B. and Sebastyen, M.M. 1957. Effect of bacterial endotoxins on the reticuloendothelial system. Federation Proc. 16: 860.

Berliner, D.L. and Dougherty, T.F. 1960. Metabolic fate of steroids. Ann. N.Y. Acad. Sc. 88: 14-29.

Biozzi, G., Benacerraf, B. and Halpern, B.N. 1953. Quantitative studies of the granulopoietic activity of the RES. II. A study of the kinetics of the granulopoietic activity of the RES in relation to the dose of carbon injected. Relationship between the weight of the organs and their activity. Brit. J. Exp. Path. 34: 441-457.

Bittner, A. 1913. Haematologishe Untersuchungen an Kaninchen bei experimenteller Trichinose, nebst einem Beitrag zur Frage der Milzextirpation. Folia Haematol. I Teil. Archiv. 15: 237-315.

Blanchard, R. and Langeron, M. 1913. Nouvelles recherches sur le paludisme des macaques. Arch. Parasit. 15: 599-607.

Blaustein, A.U. 1963. Normal and abnormal functions of the spleen. The spleen. Ed.: Blaustein, A.U. McGraw-Hill, pp. 19-44.

Bloom, W. and Fawcett, D. 1964. A textbook of histology, pp. 101-105. Eighth edition. Saunders Co.

Bouilliez, M. 1913. Nouvelle recherches experimentales sur un Plasmodium des singes. Compt. Rend. Soc. Biol. 74: 1070-1072.

Briggs, N.T. 1963. The effects of cortisone treatment on natural resistance and acquired responses of the white rat to infection with Litomosoides carinii. J. Parasit. 49: 225-230.

Browning, C.H. Cappell, D.F. and Gulbransen, R. 1934. J. Path. Bacteriol. 39: 65-74. Cited by Goble and Singer, 1960.

Bruce, J.I., von Lichtenberg, F., Schoenbechler, M.J. and Hickman, R.L. 1966. The role of splenectomy in the natural and acquired resistance of rhesus monkeys to infection with Schistosoma mansoni. J. Parasit. 52: 831-832.

Calabresi, P. and Welch, A.D. 1967. Cytotoxic drugs, hormones and radioactive isotopes., pp. 1345-1392. In "The pharmacological basis of therapeutics", 3rd ed. Goodman,L.S.R.and Gilman, A. MacMillan.

Cameron, T.W.M. 1956. Parasites and parasitism. pp. 106-111. Methuen, London, John Wiley & Son, New York.

Cameron, T.W.M. 1960. The development and experimental pathology of Echinococcus multilocularis and alveolar hydatid. Proc. "Societa Italiana di Parasitologia and the 7th Internatinal Congress of Hydatidosis" (Roma, 22 Sept. 1960) A symposium.

Campbell, W.C. 1963. Spontaneous cure in Trichuris muris infections in albino mice and its suppression by cortisone. J. Parasit. 49: 628-632.

Carpenter, P.L. 1965. Immunology and serology. 2nd Edition, Saunders.

Choquette, L.P.E., Macpherson, A.H. and Cousineau, J.G. 1962. Note on the occurrence of Echinococcus multilocularis Leuckart, 1863 in the aretic fox in Canada. Can. J. Zool. 40: 1167.

Cohn, Z.A. and Morse, S.I. 1960. Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and the consequences of particle ingestion. J. Exp. Med. 111: 667-687.

Coker, C.M. 1955a. Effects of cortisone on Trichinella spiralis infections in non-immunized mice. J. Parasit. 41: 498-504.

Coker, C.M. 1955b. Cellular factors in acquired immunity to Trichinella spiralis, as indicated by cortisone treatment of mice. J. Inf. Dis. 98: 187-197.

Coker, C.M. 1956. Effects of cortisone on cellular inflammation in the musculature of mice given one injection with Trichinella spiralis. J. Parasit. 42: 479-484.

Cross, J.H.Jr. 1960. The natural resistance of the white rat to Nematospiroides dubius and the effect of cortisone on this resistance. J. Parasit. 46: 175.

Desser, S.S. 1963. Calcium accumulation in larval Echinococcus multilocularis . Can. J. Zool. 41: 1055-1059.

Di Luzio. 1960. Reticuloendothelial involvement in lipid metabolism. Ann. N.Y. Acad. Sc. 88: 244-251.

Diggs, L.W., Sturm, D. and Bell, A. 1956. The morphology of human blood cells. Saunders, Philadelphia.

Dingle, J.H. 1941. Infectious diseases of mice. In" the biology of laboratory mouse". Ed. Snell, G.D. pp.419-429. 1941. Dover publ., New York.

Dougherty, T.F. 1952. Effects of hormones on lymphatic tissues. Phys. Revs. 32: 379-401.

Dougherty , T.F., Berliner, M.L. and Berliner, D.L. 1960. Hormonal influence on lymphocyte differentiation from RE cells. Ann. N.Y. Acad. Sc. 88: 78-82.

Draper, L.R. and Süssdorf, D.H. 1957. The serum response in intact and splenectomized rabbits following immunization by various routes. J. Inf. Dis. 100: 147-161.

Ekzemplarskaya, E.V. 1945. Med. Parazitol. Parazitar. Bolezni. 14: 34-37. Citedby Goble and Singer (1960).

Elks, E.E. and Cantrell, W. 1966. Effect of splenectomy on RES stimulation by Plasmodium berghei. J. Parasit. 51(Supplement): 36.

Ellis, E.F. and Smith, R.T. 1966. The role of the spleen in immunity with special reference to the post-splenectomy problem in infants. Pediatrics 37: 111-119.

El-Nahal, H.S. 1966. Effect of splenectomy on Plasmodium gallinaceum infections. J. Parasit. 52: 570-572.

Esch, G.W. 1967. Some effects of cortisone and sex on the biology of coenuriasis in laboratory mice and jackrabbits. Parasit. 57: 175-179.

Faulkner, R.R. and Habermann, R.T. 1957. Cellular factors in the acquired immunity to Bartonella muris. J. Inf. Dis. 101: 62-68

Fitzpatrick, J.E.P., Kennedy, C.C., McGeown, M.G., Oreopoulos, D.G., Robertson, J.H. and Soyanwo, M.A.O. 1968. A case of babesiosis in man from Northern Ireland. Nature 217: 861-862.

Florey, H.W. 1962. The influence of drugs on inflammatory processes. pp. 1029-1054. In "General pathology" 3rd ed. Florey. Saunders Co.

Florey, H.W. and Gowans, J.L. 1962. The reticuloendothelial system. The omentum. The lymphatic system. The lymphocytes. pp. 128-166. In "General pathology", 3rd Ed. Florey. Saunders Co.

Ford, W.L. 1968. The mechanism of lymphopenia produced by chronic irradiation of the rat spleen. Brit. J. Exp. Path. 49: 502-510.

Freeman, R.S., 1964. Studies on responses of intermediate hosts to infection with Taenia crassiceps (Zeder, 1800) (Cestoda). Can. J. Zool. 42: 367-385.

Friedberg, W., Neas, B.R., Faulkner, D.N. and Friedberg, M.H. 1968. Immunity to Hymenolepis nana: transfer by spleen cells. J. Parasit. 53: 895-896.

Garnham, P.SC. 1963. An introduction to protozoal immunity. pp. 3-21. In "Immunity to Protozoa". A symposium. Editors: Garnham, Pierce and Roitt. Blackwell.

Gemmell, M.A. 1962. Natural and acquired immunity. Factors inhibiting penetration of some hexacanth embryos through the intestinal barrier. Nature 194: 701-702.

Gemmell, M.A. 1966. Immunological responses of the mammalian host against tapeworm infections. IV. Specificity of hexacanth embryos in portecting sheep against Echinococcus granulosus. Immunology 11: 325-335.

Goble, F.C. and Boyd, J.L. 1957. J. Protozool. Supp. 4: 17. cited by Goble and Singer (1960).

Goble, F.C. and Singer, I. 1960. The reticuloendothelail system in experimental malaria and trypanosomiasis. Ann. N.Y. Acad. Sc. 88: 149-171.

Gonder, R. and Rodenwaldt, E. 1910. Experimentelle Untersuchungen über Affenmalaria. Centralblatt für Bakteriologie, 1 Abt. Orig. 54: 236- 240.

Gőzsy, B. and Kátó, L. 1960. Activation of capillary endotheliaum. Ann. N.Y. Acad. Sc. 88: 43-55.

Green, E.L. 1966. Biology of the laboratory mouse. 2nd. Ed. McGraw-Hill.

Halpern, B.N., Benacerraf, B. and Biozzi, G. 1953. Quantitative study of the reticuloendothelial system. I. The effect of the ingredients present in Indian ink and of substances affecting blood clotting in vivo on the fate of carbon particles administered intravenouslyiin rats, mice and rabbits. Brit. J. Exp. Path. 34: 426-440.

Ham, A.W. 1963. The structure of the spleen. In " The spleen" Editor: Blaustein, A.U. McGraw Hill, pp. 1-18.

Hawking, F. 1962. The role of the spleen in controlling the number of microfilariae. Ann. Trop. Med. Parasit. 56: 168.

Heller, J.H. 1960. Nontoxic RES stimulatory lipids. Ann. N.Y. Acad. Sc. 88: 116-121.

Heller, J.H., Meirer, R.M., Zucker, R.S., Mast, G.W. 1957. The effect of natural and synthetic estrogens on RES functions. Endocrin. 61: 235-241.

Hinz, E. 1967. Zur Chemotherapie der Echinokokkose und Zystizerkose, Grundlagen der Therapie 106: 1111-1121. (English summary).

Hnatiuk, J.M. 1966. First occurrence of Echinococcus multilocularis Leuckart, 1863 in Microtus pennsylvanicus in Saskatchewan. Can. J. Zool. 44: 493.

Hoeppli, R. 1941. Influence of splenectomy on susceptibilty of mice to infection with Taenia taeniaeformis eggs. Pro. Soc. Exp. Biol, Med. 46: 29-31.

Howard, J.G., Biozzi, G., Halpern, B.N., Stiffel, C. and Mouton, D. 1959. The effect of Mycobacterium tuberculosis (BCG) infection on the resistance of mice to bacterial endotoxin and Salmonella enteritidis infection. Brit. J. Exp. Path. 40: 281.

Hummel, K.P., Richardson, F.L. and Fekete, E. 1966. Anatomy. pp. 247-307. In "Biology of laboratory mouse" Ed. Green. McGraw-Hill.

Ingraham, J.S. 1955. Artificial radioactive antigens. III. S35-sulfarril-Azo-sheep RBC stromata. Preparation and gross distribution in normal rabbits and mice. J. Infect. Dis. 96: 105.

Inukai, T., Yamashita, J. and Mori, H. 1955. Occurrence of Echinococcus multilocularis on Rebun Island, Japan. J. Fac. Agr. Hokkaido Univ. 50: 134-139. Cited by Rausch, R.(1956).

Jackson, G.J. 1955. Effect of cortisone on Plasmodium berghei. J. Infect. Dis. 97: 152-159.

James, E. and Boyd, W. 1937. The report of a case of Echinococcus multilocularis in Winnipeg. Can. Med. Ass. J. 36: 354-356.

Jandl. J.H. and Kaplan, M.E. 1960. The destruction of red cells by antibodies in man. IV. Quantitative factors influencing the patterns of hemolysis in vivo. J. Clin. Invest. 39: 1145.

Jeffries, L. and Harris, M. 1967. Observations on the maintenance of Trichomonas vaginalis and Trichomonas foetus : the effects of cortisone and agar on enhancement of severity of subcutaneous lesions in mice. Parasit. 57: 321-334.

Jerne, N.K. and Nordin, A.A. 1963. Plaque formation in agar by single antibody-producing cells. Science 140: 405.

Kagan, I.G. 1963. Seminar on immunity to parasitic helminths. VI. Hydatid disease. Exp. Parasit. 13: 57-71.

Kiyama, T., Taylor, H.G., Williams, D.M. and Trentin, J.J. 1963. Effect of neonatal thymectomy on immunologic competence. pp.183-188. In " Conceptual advances in immunology and oncology" 1963.

Klaus, G.G. and Jones, E.W. 1968. responses in intact and splenectomized calves infected with Anaplasma marginale. J. Immunol. 100: 991-999.

Kojima, M. 1960. Morphological changes accompanying RES stimulation. Ann. N.Y. Acad. Sc. 88: 196-202.

Kritchewski, LL. 1927. Cent. Bakteriol. Abt. Orig. 104: 214-218. Cited by Goble and Singer (1960).

Larsh, J.E. Jr. 1944. The relation between splenectomy and the resistance of old mice to infection with Hymenolepis nana var. fraterna. Am. J. Hyg. 39: 133-137.

Lee, C.F. 1969. Larval Echinococcus multilocularis Leuckart, 1863 in the southern Interlake area in Manitoba. Can. J. Zool. 47: 733-734.

Leger, M. and Bouilliez. 1912. Sur un Plasmodium des singes. Passages par especes variees. Action pathogene. Compt. rend. Soc. Biol. 73: 310-313.

Leiby, P.D. 1965. Cestode in North Dakota: Echinococcus in field mice. Science 150: 763.

Leiby, P.D.,Lubinsky,G. and Galaugher, W. 1969. Studies on sylvatic Echinococcosis. II. The occurrence of Echinococcus multilocularis, Leuckart, 1863 in Manitoba. Can. J. Zool. 47: 135-138.

Leiby, P.D. and Nickel, M.P. 1968. Studies on sylvatic echinococcosis. II. Ground beetle transmission of Echinococcus multilocularis, Leuckart, 1863 to deer mice, Peromyscus maniculatus (Wagner). J. Parasit. 54: 536-537.

Leiby, P.D. and Olsen, O.W. 1964. The cestode Echinococcus multilocularis in foxes in North Dakota. Science 145: 1066.

Leuckart, R. 1863. Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten. Ein Hand- und Lehrbuch für Naturforscher and Aerzte. Leipzig and Heidelberg. 766p.

Lubinsky, G. Unpublished data. Personal communication.

Lubinsky, G. 1960a. Note on the use of McIlvaine's buffer solutions in staining blood protozoa. Can. J. Zool. 38: 437-438.

Lubinsky, G. 1960b. The maintenanee of Echinococcus multilocularis sibiricensis without the definitive host. Can. J. Zool. 38: 149-151.

Lubinsky, G. 1960 c. A vegetatively propagated strain of larval Echinococcus multilocularis. Can. J. Zool. 38: 1117-1125.

Lubinsky, G. 1964. Growth of vegetatively propagated strain of larval Echinococcus multilocularis in some strains of Jackson mice and in their hybrids. Can. J. Zool. 42: 1099-1103.

Lubinsky, G. 1966. Accelerating growth of a vegetatively propagated strain of larval Echinococcus multilocularis Leuckart, 1863. Can. J. Zool. 45: 175-181.

Lubinsky, G. 1969. Gerbils as a primary screen in experimental chemotherapy of infection with larval Echinococcus multilocularis. J. Parasit. 55: 224-225

Lubinsky, G. and Desser, S.S. 1963. Growth of vegetatively propagated strain of larval Echinococcus multilocularis in C57/J, B6AF$_1$, and A/J mice. Can. J. Zool. 41: 1213-1216.

Lubinsky, G. and Galaugher, W. 1967. Inhibition of the growth of Echinococcus multilocularis cysts by cyclophosphamide. Can. J. Zool. 45: 1302-1303.

Lukashenko, N.P. 1966. Comparative study of the genesis of the cysts of Alveococcus multilocularis (Leuckart, 1863) Abuladze, 1959 in some mammals from a consideration of host-parasite relationship. Med. Parasitol. Parazitarn Bolezni. 35:474-481. Cited by Helminthological abstract 37: 1754. (English summary).

Lurie, M.B. 1960. The RES, cortisone, and thyroid function. The relation to native resistance to infection. Ann. N.Y. Acad. Sc. 88: 83-98.

Machado, E., Lozzio, B.B. and Royer, M. 1968. Cellular modifications of the reticuloendothelial system submitted to different stimuli. J. RE Society. 5: 297-314.

Magee, P.N. and Palmer, A.A. 1953. The absortion of particles from the peritoneal cavity in rats and mice treated with cortisone. Brit. J. Exp. Path. 34: 458-463.

Mankau, S.K. 1955. Studies on Echinococcus alveolaris (Klemm, 1883), from St. Lawrence Island, Alaska. III. The histopathology caused by the infection of E. alveolaris in white mice. Am. J. Trop. Med. Hyg. 5: 872-880.

Mankau, S.K. 1957. Studies of Echinococcus alveolaris (Klemm, 1883) from St. Lawrence Island, Alaska. I. Histogenesis of alveolar cysts in white mice. J. Parasit. 43: 153-159.

Marmont, A. and Biassoni, P. 1962. The effect of antiphlogistic steroids in selected cases of non-immunologic haemolytic anaemia. Clinical and haematological observations. Possible role of the reticuloendothelial system. Role du système réticulo-endothélial dans l'immunité anti-bactérienne et antitumorale. pp. 497-525. Colloques Internationaux du Centre National de la Recherche Scientifique.

Mathies, A.W. Jr. 1962. Certain aspects of the host-parasite relationships of Aspicularis tetraptera, a mouse pin-worm. III. Effect of cortisone. J. Parasit. 48: 244-248.

Menkin, V. 1953. The significance of the accumulation of cortisone in an inflamed area. Brit. J. Exp. Path. 34: 412-419.

Miller, J.F.A.P. 1961. Immunological function of the thymus. Lancet. 2: 748-749. Cited by Kiyama et al (1963).

Miller, T.A. 1966. The effect of synthetic corticosteroids upon the susceptibility of dogs to infection with human hookworms Necator americanus. J. Parasit. 52: 285-290.

Morseth, D.J. and Soulsby, E.J.L. 1969. Fine structure of leucocytes adhering to the cuticle of Ascaris suum larvae. II. Polymorphonuclear leucocytes. J. Parasit. 55: 1025-1034.

Newberne, P.M., Hunt, C.E. and Young, V.R. 1968. The role of diet and reticuloendothelial system in the responses of rats to Salmonella typhimurium infection. Brit. J. Exp. Path. 49: 448-457.

Nicol, T., Cordinley, J., Charles, L., McKelvie, P., Bilbey, D. 1962. Effect of reticuloendothelial stimulation on experimental infection. pp.165-187. Role du système réticuloendothélial dans l'immunité anti-bactérienne et antitumorale. Centre National de la Recherche Scientifique.

Nooruddin and Ahmed, S.S. 1967. The effects of splenectomy on parasitc infections and the role of the spleen in filariasis. — a brief appraisal of our present knowledge. J. Trop. Med. Hyg. 70: 229-232.

Ogilvie, B. 1965. Use of cortisone derivatives to inhibit resistance to Nippostrongylus brasiliensis and to study the fate of the parasites in resistant hosts. Parasit. 55: 723-730.

Ogilvie, B.M. and Jones, V.E. 1967. Reagenic antibodies and immunity to Nippostrongylus brasiliensis in the rat. I. The effects of thymectomy and splenectomy. Parasit. 57: 335-349.

Ohbayashi, M. and Sakamoto, T. 1966. Studies of echinococcosis. XVII. Sex difference in resistance to infection with Echinococcus multilocularis in uniform strains of mice. Jap. J. Vet. Res. 14: 66-70.

Oliver, L. 1962. Studies on natural resistance to Taenia taeniaeformis in mice. II. The effect of cortisone. J. Parasit. 48: 758-762.

Olsen, O.W. 1962. Animal parasites: their biology and life cycles. Minneapolis, Burgess Publ. Co.

Olson, L.J. 1959. The cellular response of white rats to Litomosoides carinii larvae as influenced by cortisone, age and previous infection. J. Parasit. 45: 519-532.

Paraskevas, F. 1969. Notes on hematology and chemotherapy: immunological competence. Medical College, University of Manitoba. October 24, 1969. (Unpublished).

Parker, J.C. 1961. Effect of coetisone on the resistance of the guinea pig to infection with the rat nematode Nippostrongylus brasiliensis. Exp. Parasit. 11: 380-390.

Plimmer, H.G. and Bradford, J.R. 1899. Nature 60: 309-311. Cited by Goble and Singer (1960).

Rausch, R. 1956. Studies on the helminth fauna of Alaska. XXX. The occurrence of Echinococcus multilocularis Leuckart, 1863 on the mainland of Alaska. Am. J. Trop. Med. Hyg. 5: 1086-1092.

Rausch, R.L. 1968. Taxonomic characters in genus Echinococcus (Cestoda: Taeniidae). Bull. Wld. Hlth. Org. 39: 1-4.

Rausch, R. and Schiller, E.L. 1951. Hydatid disease (echinococcosis) in Alaska and the importance of rodent intermediate hosts. Science 113: 57-58.

Rausch, R. and Schiller, E.L. 1954. Studies on the helminth fauna of Alaska. XXIV. Echinococcus sibiricensis n.p. from St. Lawrence Island. J. Parasit. 40: 659-662.

Rausch, R. and Schiller E.L. 1956. Studies on the helminth fauna of Alaska. XXV. The ecology and public health significance of Echinococcus sibiricensis Rausch & Schiller, 1954 on St. Lawrence Island. Parasit. 46: 396-419.

Rebuck, J.W., Boyd, C.B.and Riddle, J.M. 1960. Skin windows and the action of the RES. Ann. N.Y. Acad. Sc. 88: 30-42.

Reed, N.D. and Jutila, J.W. 1967. Wasting disease induced with cortisol acetate. Immunology 99: 238-245.

Riley, V. 1960. Adaptation of orbital bleeding technique to rapid serial blood studies. Proc. Soc. Exp. Biol. Med. 104: 751-754.

Rindani, T.H. and Selye, H. 1953. Inflammation as influenced by topical blockade of the RES: an example of "local cross sensitization". Brit. J. Exp. Path. 34: 674-680.

Ritterson, A.L. 1959. Innate resistance of species of hamsters to Trichinella and its reversal by cortisone. J. Infect. Dis. 105: 253-266.

Ritterson, A.L. and Concannon, J. 1968. Comparative effects of cortisone and ACTH on golden and Chinese hamsters infected with Trichinella spiralis. J. Parasit. 54: 822.

Roberts, O.J. 1954. The effect of cortisone on Plasmodium berghei infection. Parasit. 44: 58-64.

Rowley, D.A. 1950. Effect of splenectomy on the formation of circulating antibody in the adult male albino rat. J. Immunol. 64: 289.

Russel, E.S. and Bernstein, S.E. 1966. Blood and blood formation. pp.351-372. In " Biology of laboratory mouse", 2nd. Ed. Green, E.L., McGraw-Hill.

Sadun, E.D., Norman, L., Allan, D.S. and King, N.M. 1957. Observations on the susceptibility of cotton rats to Echinococcus multilocularis Leuckart, 1863. J. Infect. Dis. 100: 273-277.

Sakamoto, T., Yamashita, J., Ohbayashi, M. and Orihara, M. 1965. Studies on echinococcosis. XVI. Effects of drugs upon scolieces and daughter cysts of Echinococcus multilocularis in vitro. Jap. J. Vet. Res. 13: 127-135.

Schiller, E.L. 1954a. Studies on the helminth fauna of Alaska. XXVI. Some observations on the cold resistance of eggs of Echinococcus sibiricensis Rausch and Schiller, 1954. J. Parasit. 41: 578-582.

Schiller, E.L. 1954b. An experimental study of blowfly (Phormia regina) transmission of hydatid disease. Exp. Parasit. 3: 161-166.

Schiller, E.L. 1965. Observations on some host-parasite relationships among arctic wildlife. In "Host-parasite relationships" Ed. McCauley, J.E. Proceedings of the 26th Annual Biology Colloquium, April 23-24, 1965. Oregon State University Press.

Scholtens, R.G., Braff, E.H. and Healy, G.R. 1968. A case of babesiosis in man in the U.S.A. Am. J. Trop. Med. Hyg. 17: 810-813.

Schuurmam, C,J. and Schuurman-Ten Bokkel Huinink, A.M. 1929. Mededeel. Dienist Volksgezondheid Ned. Indie. 18: 469-497. Cited by Goble and Singer (1960).

Schwabe, C.W. 1959. Host-parasite relationships in echinococcosis. I. Observations on the permeability of the hydatid cysts wall. Am. J. Trop. Med. Hyg. 8: 20-28.

Schwabe, C.W., Schinazi, L.A. and Kilejian, A. 1959. Host-parasite relationships in echinococcosis. II. Age resistance to secondary echinococcosis in white mouse. Am. J. Trop. Med. Hyg. 8: 29-36.

Selye, H. and Horava, A. 1953. Third annual report on stress. ACTA, Inc. Med. Publ., Montreal, Canada.

Sherman, I.W. and Puble, J.A. 1967. Virulent Trypanosoma lewsi infections in cortisone-treated rats. J. Parasit. 53: 258-262.

Sigurdson, E. 1969. The effects of Echinococcus multilocularis cyst growth on the behavior of LD_1F_1 hybrid mice. Project report for Course 22.444. University of Manitoba. 9 pages. (Unpublished).

Silverman, P.H. 1965. A concept of host-parasite immunologic relationships. pp. 117-126. In "Host-parasite relationships" Ed. McCauley, J.E. Proseeding a of the 26th annual biology colloquium. Oregon State University Press.

Skrabalo, Zdenko, and Deanovic, Zivan, 1957. Piroplasmosis in man. Report on a case. Doc. de Med. Geogr. et Trop. 9: 11-16.

Slattery, L. 1963. Surgical management of splenomegaly. In"the spleen" pp. 207-228. Ed. Blaustein, A.U., McGraw-Hill.

Smyth, J.D. 1964. The bidogy of the hydatid organisms. Advances in Parasit. 2: 169-219.

Smyth, J.D. 1969. The biology of the hydatid organisms. Advances in Parasit. 7: 327-347.

Smyth, J.D. and Smyth, M.M. 1964. Natural and experimental hosts of Echinococcus granulosus and E. multilocularis with comments on the genetics of speciation in the genus Echinococcus. Parasit. 54: 493-514.

Snell, J.F. 1960. The reticuloendothelial system. I. Chemical methods of stimulation of the RES. Ann. N.Y. Acad. Sc. 88: 56-77.

Spain, D.M., Molomut, N. and Haber, A. 1950. Biological studies on cor cortisone in mice. Science 112: 335-337.

Stirling, G.A. and Kakkar, V.V. 1969. Cells in the circulating blood capable of producing connective tissue. Brit. J. Exp. Path. 50: 51-55.

Tada, I. 1967. The effect of worm burden and cortisone on Paragonimus infeetion in albino rats. Jap. J. Parasit. 16: 51-57. from Helmin. Abs. 37: 1905 (Abstract).

Taliaferro, W.H. 1940. The mechanism of immunity to metazoan parasites. Am. J. Trop Med. Hyg. 20: 169-182.

Taliaferro, W.H. 1948. The role of the spleen and the lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. I. Acquired immunity and phagocytosis. J. Infect. Dis. 83: 164-180.

Taliaferro, W.H. 1956. Functions of the spleen in immunity. Am. J. Trop. Med. Hyg. 5: 391-410.

Taliaferro, W.H. 1963. Cellular and humoral factors in immunity to Protozoa. "Immunity to Protozoa." A symposium of the British society for immunology. Editors: Garnham, P.C.C., Pierce, A.E. and Roitt, I. Blackwell Publ.

Taliaferro, W.H., Cannon, P.R. and Goodloe, S. 1931. The resistance of rats to infection with Trypanosoma lewisi as affected by splenectomy. Am. J. Hyg. 14: 1-37.

Taliaferro, W.H. and Kelsey, F.E. 1948. The role of the spleen and lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. II. Quinine blood levels. J. Infect. Dis. 83: 181-199.

Taliaferro, W.H. and Pavlinova, Y. 1936. The course of infection of Trypanosoma duttoni in normal and in splenectomized and blockaded mice. J. Parasit. 22: 29-41.

Thatcher, V.E. and Sousa, O.E. 1967. Echinococcus oligarthrus (Diesing, 1863) from a Panamanian jaguar (Felis onca L.). J. Parasit. 53: 1040.

Thompson, J.H.Jr. 1954. Host-parasite relationships of Schistosoma mansoni. Exp. Parasit. 3: 140-160.

Thompson, P.E., McGinty, D.A., Bush, D.L. and Wilson, M.L. 1968. J. Inf. Dis. 83: 23-32. Cited by Goble and Singer (1960).

Todorovic, R., Ferris, D. and Ristic, M. 1967. Roles of the spleen in acute plasmodial and babesial infections in rats. Exp. Parasit. 21: 354-372.

Torre, C.M. and Leiken, S.L. 1959. Tissue inflammatory cytology in clinical states. Am. J. Clin. Path. 32: 335.

Trager, W. 1941. Am. J. Hyg. 34: 141-144. Cited by Goble and Singer(1960).

Travis, R.H. and Sayers, G. 1967. Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs. pp. 1608-1648. In "The pharmacological basis of therapeutics". Ed. Goodman, L.S. and Gilman, A. 3rd Ed.

Verster, A.J.M. 1965. Review of Echinococcus species in South Africa. Onderstepvort J. Vet. Res. Edit. Jansen, B.C. 32: 7-118. Government Printer, Prestoria, South Africa.

Villarejos, V.M. 1962. Cortisone and experimental amoebiasis in the rat. J. Parasit. 48: 194.

Virchow, R. 1855. Die multiloculare, ulcerirende Echinokokkengeschwulst der Leber. Verhandl. Phys.Med. Gesellsch. Wurzburg. 6: 84-95. Cited by Cameron, T.W.M.(1960).

Vogel, H. 1957. Uber den Echinococcus multilocularis Süddeutschlands. I. Das Bandwurmstadium von Stammen menschlicher und tierischer Herkunft Ztschr. Tropenmed. u. Parasit. 8: 404-454. (English summary).

von Berenberg-Gossler, H. 1909. Arch. Protistenk. 16: 245-280. Cited by Goble an Singer (1960).

Webster, G.A. and Cameron, T.W.M. 1961. Observations on experimental infections with Echinococcus in rodents. Can. J. Zool. 39: 877-891.

Weinmann, C.J. 1968. Effects of splenectomy and neonatal thymectomy on acquired immunity to dwarf tapeworm Hymenolepis nana. Exp. Parasit. 22: 68-72.

Weinmann, C.J. and Hunter, G.W. 1960. III. Studies on schistosomiasis. XIV. Effects of cortisone upon the Schistosoma mansoni in mice. Exp. Parasit. 9: 239-242.

Weinmann, C.J. and Rothman, A.H. 1961. Effects of natural stressors and cortisone upon acquired resistanceto Hymenlepis nana in mice. J. Parasit. 47: supplement 133, section 2. Abstract.

Weinstein, P.P. 1953. The effects of cortisone on the development of immune response in the white rat to Nippostrongylus muris. J. Parasit. 39(supple.4-2): 35.

Wissler, R.W., Fitch, F.W. and La Via, M.F. 1960. The RES in antibody formation. Ann. N.Y. Acad. Sc. 88: 134-148.

Wright, H.D. 1927. Experimental pneumococcal septicemia and anti-pneumococcal immunity. J.Path. & Bact. 30: 185-252. (after Carpenter, 1965).

Yamashita, J. 1960. On the susceptibility and histogenesis of Echinococcus multilocularis in the experimental mouse, with the state of echinococcosis in Japan. Parasitologia 2: 399-406.

Yamashita, J.M. Ohbayashi, Y. Kitamura, K. Suzuki and Okugi, M. 1958 Jap. J. Vet. Res. 6: 135. Cited by Yamashita et al (1963).

Yamashita, J., Ohbayashi, M., Sakamoto, T. and Orihara, M. 1963. Studies on echinococcosis. XIV. Further observationa on the dif difference of susceptibility to Echinococcus multilocularis among uniform strains of the mouse. Jap. J. Vet. Res. 11: 50-70.

Zaiman, H., Ingalls, J.W. Jr., and Villaverde, H. 1962. Mortality and survival time of trichinized mice treated with cortisone acetate. Exp. Parasit. 12: 418-422.

Zuckerman, A. and Yoeli, M. 1954. Age and sex as factors influencing Plasmodium berghei infections in intact and splenectomized rats. J. Inf. Dis. 94: 225-236.

APPENDIX I

Glossary

Athreptic immunity	an immunity based on the scarcity of nutrients necessary for the development of a certain parasite.
Atropghy	decrease in size of a tissue, organ, or part after full development has been attained.
Concentration Index	a quantitative measure of the dose of Echinococcus cysts used for infection. It is defined as the numerical value of the ratio: $\frac{\text{cyst material in c.c.}}{\text{cyst material in c.c. + saline in c.c.}}$
Cortone	the trade name of cortisone acetate (Merck and Dohme, Canada)
Day O	the day of infection
DPI	days post-infection
Hydatid cyst	larval form of Echinococcus. A fertile cyst contains protoscoleces. A sterile cyst (syn. acephalic cyst) does not contain protoscoleces.
Mortality rate	accumulative death toll of experimental animals expressed in percentages. Here it is a minimum estimate since the possible toll of mice autopsied earlier is not taken into consideration in subsequent enumerations.
Protoscolex	The precursor of the adult scolex in developing Echinococcus cysts.
Resistant host	the term may be used in different senses: (1) to designate host in which the infection rate is zero or very small; (2) to designate host in which the rate of growth of the parasite is very slow. In the present paper the term "resistant" is mostly used in the second sense.
Susceptible host	the term "susceptible host " may be used in different senses: (1) to designate host in which the rate of growth of the parasite is very fast. In the present paper the term "susceptible" is mostly used in the second sense.

APPENDIX II

Chemotherapy* of larval Echinococcus multilocularis by six drugs in LD_1F_1 mice

Drug	Exp. No.	Group	Mice autopsied	Dosage mg/ Kg	Mode of Injection	Days of Infection	Gross Wt. gm.	Cyst wt. gm.	t	p
Vincristine	1	Control	14 ♀	-	-	50	28.1	7.91± 0.85		
		Exp.	14 ♀	10 x 2	I.V.	50	27.2	6.80± 0.69	1.02	>0.05
Vinblastine	2	Control	19 ♀	-	-	52	-	4.44± 0.89		
		Exp.	24 ♀	10 x 2	I.V.	52	-	3.36± 0.45	1.11	>0.05
	3	Control	24 ♀	-	-	49	25.0	5.24± 0.72		
		Exp.	18 ♀	5 x 5	I.V.	49	22.9	6.16± 0.52	1.03	>0.05
Colchicine	4	Control	27 ♀	-	-	49	-	10.06± 1.06		
		Exp.	20 ♀	1 x 7	S.C.	49	-	9.64± 0.94	0.30	>0.05
Colcemide	5	Control	9 ♂	-	-	77	32.6± 0.9	15.49± 0.76		
		Exp. #1	7 ♂	0.5x 6	S.C.	77	31.1± 0.8	14.50± 0.80	0.89	>0.05
		Exp. #2	8 ♂	1 x 6	S.C.	77	29.8± 0.8	13.02± 0.53	2.67	<0.05
		Exp. #3	4 ♂	2 x 5	S.C.	77	27.1± 2.1	10.45± 0.68	4.05	<0.01
	6	Control	22 ♀	-	-	30	24.9	7.68± 0.77		
		Exp.	12 ♀	1 x 4	S.C.	30	24.4	6.23± 0.51	1.31	>0.05
Hetrazan	7	Control	27 ♀	-	-	49	-	10.06± 1.06		
		Exp.	26 ♀	300 x 9	S.C.	49	-	9.25± 0.62	0.67	>0.05
Dactinomycin	8	Control	9 ♂	-	-	30	31.8	2.04± 0.21		
		Exp.	15 ♂	0.17x 2	I.V.	30	31.8	1.16± 0.18	3.19	<0.01
	9	Control	10 ♀	-	-	38	25.8	6.10± 0.56		
		Exp.	15 ♀	0.17x 4	I.V.	38	25.2	4.45± 0.48	2.24	<0.05
	10	Control	20 ♀	-	-	49	28.7	5.73± 0.67		
		Exp. #1	18 ♀	0.35x 5	I.V.	49	25.0	3.77± 0.64	2.13	<0.05
		Exp. #2	19 ♀	0.35x 5	S.C.	49	25.3	3.03± 0.48	3.29	<0.01
		Exp(1+2)	37 ♀	0.35x 5	(Pooled)	49	25.2	3.39± 0.39	3.03	<0.01

* Courtesy of Dr. G. Lubinsky.

APPENDIX II

Correlation of spleen weight with the weight of Echinococcus cysts in six different types of mice

Mice *	Colour	Age months	Concentration Index **	Survival Ratio	Autopsy DPI #	% Infection	Gross Wt. at Autopsy gm.	Cyst Wt. x gm.	Spleen Wt. y gm.	Correlation Coefficient r_{xy}
LD_1F_1	brown	3	0.21	19/20	60	100	28.3	6.74± 1.09	0.53 ± 0.03	- 0.11
CE	dirty white	3	0.21	19/20	60	100	28.2	2.38± 0.30	0.24 ± 0.03	0.86
AKR	white	2½	0.21	20/20	60	100	26.7	1.17± 0.16	0.19 ± 0.02	0.66
B10.D_2-New/Sn	black	2½	0.21	17/19	60	100	22.3	1.06± 0.24	0.26 ± 0.04	0.86
SJL	white	2½	0.21	15/16	60	100	21.7	0.74± 0.19	0.22 ± 0.02	0.74
C3HeB/Fe	shiny brown	3	0.21	19/20	60	95	26.7	0.22± 0.04	0.22 ± 0.02	0.84

* all females
** a measure of the dose of cyst material used for infecting mice
\# days post-infection

APPENDIX IV

Lines of further research

I recommend that further research work on Echinococcus multilocularis in our laboratory should follow these three directions.

(1) General approach. A reversal to infect rodents with onchospheres of E. multilocularis is highly adviseable to simulate natural infection.

(2) Academic interest. The intraperitoneal histopathological picture of mice infected with intraperitoneal Echinococcus cysts should be studied. Deoxycorticosterone acetate (DCA) is prophlogistic and is expected to inhibit the growth of larval E. multilocularis cysts, at least in the early stages of infection. Studies on serological picture in $B6D2F_1$ mice, 100 days after infection, may prove the presence of a delayed acquired immunit to E. multilocularis multilocularis in these animals.

(3) Therapeutic research. Emphasis should be placed on chemotherapy rather than on vaccination. The principle used in chemotherapy should be on selective toxicity rather than a stimulation of cellular responses. In vitro culture method which avoids immunological complications and shortens the period of experiments may prove to be useful. Sakamoto et al (1965) did preliminary work on E. multilocularis along this line.