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THE BIOLOGY AND HOST-PARASITE RELATIONSHIP OF THE CAT LIVER FLUKE, *PLATYNOSOMUM CONCINNUM* (BRAUN, 1901)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTORATE OF PHILOSOPHY

IN ZOOLOGY

AUGUST 1975

By

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ABSTRACT

Various aspects of the host-parasite relationship between the liver fluke (Platynosomum concinnum syn. fastosum) and its natural, definitive host the cat (Felis catus domesticus) were studied. Clinical, pathologic, hematologic, serum biochemical, enzymatic and immunologic parameters were used to monitor the changes during the course of infection. Experimental infection was induced in conditioned cats by the oral administration of P. concinnum infective stages. The animals were then observed and studied for nine months.

Mild clinical signs of inappetence, anorexia, and lethargy were noted in 30% of the cats within a period of 7-12 weeks post-infection. Clinical symptoms were observed during the time required for the juvenile P. concinnum to become established and reach maturity in the file ducts. As the parasite matured and increased in size, the bile ducts became dilated and intermittent biliary stasis was noted. Pathologic alterations resulted in a massive infiltration of lymphocytes, eosinophils and polymorphonuclear leucocytes around the bile ducts, as well as the release of liver parenchymal cell transaminase enzymes. Thus serum transaminase levels (SGOT and SGPT) became elevated.

Once the flukes reached sexual maturity (8-10 weeks post-infection) and the major ducts had undergone pathologic damage, fibrosis tended to "wall-in" the fluke. Serum transaminase enzyme levels then returned within normal ranges 4 months after the initial experimental P. concinnum infection. Autoradiographic determination of the regeneration capacity of the liver cells and enzymatic analysis of extracts of

hepatic tissue for peroxidase, catalase, glutamic-pyruvic acid transaminase, glutamic-oxaloacetic acid transaminase, d-amino acid oxidase, l-α-hydroxyacid oxidase, and uricase suggest additional damage to parenchymal cells did not occur.

The host responded immunologically by producing humoral antibody which was detected in the bile. The immunoglobulin (IgG) was not cytotoxic to P. concinnum, but bound to its surface and functioned as a blocking antibody.

Six to nine months after experimental infection T and B lymphocyte analysis (using both rosette formation and immunofluorescent staining technics) and lymphocyte blast transformation (using pokeweed mitogen, phytohemagglutinin, and concanavalin A) indicated P. concinnum had not immunologically compromised, i.e. immunosuppressed, its feline host. Passive transfer of immune serum or previous infection with P. concinnum had no protective value when cats received an oral experimental challenge dose of P. concinnum infective stages; however, cats receiving sensitized lymphocytes from a donor animal prior to challenge had a 65% reduction in fluke burden at necropsy. Cell mediated immunity therefore seems to be involved in regulating the initial stages of P. concinnum infection.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF TABLES	vii
LIST OF ILLUSTRATIONS	x
LIST OF PLATES	xiii
INTRODUCTION	1
MATERIALS AND METHODS	16
RESULTS	50
DISCUSSION AND CONCLUSION	133
ADDENDUM	153
PLATES	167
APPENDIX A	179
APPENDIX B	186
REFERENCES	235

LIST OF TABLES

Table		Page
1	Worldwide Prevalence of P. concinnum	4
2	Symptoms Associated with Platynosomiasis in Naturally Infected Cats	6
3	Infection and Bleeding Schedule for Experimentally Infected Cats	20
4	Anatomical Distribution of P. concinnum within the lobes of the liver of Experimentally Infected Cats	82
5	The Prevalence of Selected Feline Parasites on Oahu, 1974	108
6	Enzyme Levels in Liver Tissue of Normal and Parasitized Cats	112
7	Radioimmunoabsorbance Assay for the Presence of Feline IgG on the Surface of P. concinnum	117
8	The Number of T and B Lymphocytes as Determined by Rosette Formation in Control and Parasitized Cats	119
9	The Number of T and B Lymphocytes as Determined by Immunofluorescent Staining in Control and Parasitized Cats	120
10	The Effect of Mitogens on Feline Lymphocyte Blast Transformation	122
11	Fluke Burden of Control, Serum and Lymphocyte Recipient Cats	128
12	The Distribution of Flukes in the Hepatic Lobes of Cats Receiving Treatment Prior to Primary P. concinnum Infection	130
13	Summary of the Size of Flukes Recovered from Cats Treated Prior to P. concinnum Infection	129
14	Surface Area of P. concinnum Recovered at Necropsy from Untreated, Serum and Lymphocyte Recipient Cats	131
15	Rosette Formation of Peripheral Blood Lymphocytes 50 Days Post-Experimental Infection	132

Table		Page
16	The Infection and Necropsy Schedule for Alternate Definitive Hosts	156
17	Hematologic Values for Swiss White Mice during Experimental Platynosomiasis	159
18	Peroxisomal Enzyme Activity for Control and Parasitized Swiss White Mice	163
19	Weight of Individual Cats Previous to Infection	180
20	Weight Changes During the Course of Infection for Individual Cats	181
21	Weight Changes During the Course of Infection for Group A, B and C Cats	184
22	The Percentage of Eosinophils Present in the Serum of Experimentally Infected Cats	187
23	The Number of Serum Eosinophils Present During the Course of Infection	189
24	Serum Glutamic Pyruvic Transaminase Values for Group A, B and C Cats	191
25	Serum Glutamic Oxaloacetic Acid Transaminase Values for Group A, B and C Cats	193
26	Serum Bilirubin Values for Group A, B and C Cats	195
27	Serum Alkaline Phosphatase Values for Group A, B and C Cats	197
28	Icterus Index Values for Group A, B and C Cats	199
29	Hematocrit Values for Group A, B and C Cats	201
30	Total Erythrocyte Count for Group A, B and C Cats	203
31	Blood Hemoglobin Content for Group A, B and C Cats	205
32	Sedimentation Rate for Group A, B and C Cats	207
33	Mean Cell Volume for Group A, B and C Cats	209
34	Mean Cell Hemoglobin Values for Group A, B and C Cats	211

Table		Page
35	Mean Cell Corpuscular Hemoglobin Content for Group A, B and C Cats	213
36	Total Serum Protein Concentration for Group A, B and C Cats	215
37	Serum Albumin Concentration for Groups A, B and C Cats	217
38	Serum Globulin Concentration for Group A, B and C Cats	219
39	Serum Inorganic Phosphate Levels for Group A, B and C Cats	221
40	Serum Inorganic Calcium Levels for Group A, B and C Cats	223
41	Serum Chloride Levels for Group A, B and C Cats	225
42	Total Leucocyte Count for Group A, B and C Cats	228
43	Blood Urea Nitrogen Values for Group A, B and C Cats	231
44	Serum Glucose Values for Group A, B and C Cats	233

LIST OF FIGURES

Figure		Page
1	Metabolic Relationship of Peroxisomal Enzymes in Hepatic Tissue	12
2	The Isolation and Purification of Feline IgG	41
3	Schedule for *in vivo* Study Assessing the Role of Cell Mediated Immunity in Platynosomiasis	49
4	Signs and Symptoms of Five Group C Cats with Experimentally Induced *P. concinnum* Infection	51
5	The Average Weight of Male and Female Cats Following *P. concinnum* Infection	52
6	Eosinophilia in Male Cats Following *P. concinnum* Infection	54
7	Eosinophilia in Female Cats Following *P. concinnum* Infection	55
8	SGPT Levels in Male Cats During *P. concinnum* Infection	57
9	SGPT Levels in Female Cats During *P. concinnum* Infection	58
10	SGOT Levels in Male Cats During *P. concinnum* Infection	59
11	SGOT Levels in Female Cats During *P. concinnum* Infection	60
12	Serum Icterus Index and Total Bilirubin Values in Group C Cats	61
13	PCV, RBC, Hemoglobin Content and Sedimentation Rate in Group B and C Cats	63
14	MCV, MCH and MCHC Values for Group B and C Cats	64
15	Total Serum Protein, Albumin, and Globulin Values for Experimentally Infected Cats	65
16	The Concentration of Serum Ions in Experimentally Parasitized Cats	66

Figure		Page
17	White Blood Cell, Glucose and BUN Studies in Experimentally Infected Cats	67
18	Hematologic Values for Cat No. 28	68
19	Hematologic Values for Cat No. 29	70
20	Number of Ova Per Gram of Feces During P. concinnum Infection	72
21	Liver Pathologic Changes During Experimental Platynosomiasis	75
22	Summary of Pathologic Findings Recorded at Necropsy . .	80
23	Percentage of Flukes Recovered at Necropsy During the Course of Infection	81
24	Histopathologic Sections of Hepatic Tissue Before and Seven Days after P. concinnum Infection	84
25	Histopathologic Sections of P. concinnum Within a Major Bile Duct Twenty-six Days Post-Infection	85
26	Histopathologic Sections of Liver Twenty-six Days Post-Infection	87
27	Histopathologic Sections of Hepatic Tissue Six Weeks Post-Infection	88
28	Histopathologic Sections of Hepatic Tissue Six Weeks Post-Infection	89
29	Histopathologic Sections of Hepatic Tissue Four Months Post-Infection	90
30	Histopathologic Sections of Hepatic Tissue Nine Months Post-Infection	92
31	Infective Stage Isolated From Bile Ducts of Bufo marinus	93
32	Growth Rate of P. concinnum in the Cat	95
33	The Developmental Stage of P. concinnum Present in the Liver of Cats Fourteen Days Post-Infection	96

Figure		Page
34	The Developmental State of P. concinnum Present in the Liver of Cats Nineteen Days Post-Infection	98
35	The Developmental Stage of P. concinnum Present in the Liver of Cats Twenty-six Days Post Infection	99
36	The Developmental Stage of P. concinnum Present in the Liver of Cats Forty-two Days Post-Infection	100
37	The Developmental Stage of P. concinnum Present in the Liver of Cats Sixty-four Days Post-Infection	101
38	Autoradiographic Study of the Regeneration Capacity of Liver Tissue During P. concinnum Infection	103
39	An Autoradiograph of Hepatic Parenchymal Cells Labeled with Tritiated Thymidine	104
40	Hepatic Enzyme Levels During the Course of P. Concinnum Infection	105
41	Peroxisomal Enzyme Levels in Hepatic Tissue During the Course of P. concinnum Infection	106
42	The Distribution of Parasite Burden in Adult Cats . . .	110
43	SGOT and SGPT Activity Levels in a Random Population of Cats	111
44	Cellulose Acetate Strip Electrophoresis of Bile	114
45	Polyacrylamide Gel Electrophoresis of Bile	115
46	Response of Feline Lymphocytes to Various Concentrations of Mitogens	121
47	Summary of Hematologic Values for Untreated Cats	125
48	Summary of Hematologic Values for Serum and Lymphocyte Recipient Cats	126
49	Developmental Sequence of P. concinnum in the Swiss White Mouse	161

LIST OF PLATES

Plate		Page
1	The Infective Stage of Platynosomum concinnum Recovered from the Liver of the Toad Bufo marinus	168
2	Juvenile Fluke Recovered from the Liver of a Cat Seven Days after Infection	170
3	Maturing Fluke Nineteen Days after Infection	172
4	The Developmental Stage of the Fluke Twenty-one Days after Infection	174
5	Reproductive Organs of Platynosomum concinnum Six Weeks after Infection	176
6	Some Important Structures of Adult P. concinnum	178

INTRODUCTION

Platynosomum concinnum, Braun, 1901, is a digenetic trematode found in the gall bladder and bile ducts of the liver of cats in the tropics. This species was first reported in Hawaii in 1962 (Ash, 1962). Surveys on the prevalence of cat liver fluke infection indicate a current incidence in Hawaii of over 40% (Palumbo et al., 1974). Because of the paucity of information in the literature concerning platynosomiasis, veterinarians and feline researchers throughout the world are uninformed about this important disease.

The original description of P. concinnum (Platyhelminthes: Dicrocoeliidae) was made by Braun in 1901. In 1910 Kossack described another liver fluke, Platynosomum fastosum, from the gall bladder of the civet cat, Felis minita Tenn. A third biliary fluke, Platynosomum planicipitis, (Cameron, 1928) has also been reported. Unless additional information concerning the biology and life cycle of these parasites indicate otherwise, based on morphological considerations; P. concinnum, P. fastosum, and P. planicipitis should be considered as the same liver fluke (Purvis, 1931).

In the original description, P. concinnum is described as a biliary parasite approximately 4.6-5.8 mm x 1.8-2.0 mm (Kossack, 1910). The suckers are of equal size (0.38 mm in diameter) spaced 1.4 mm apart. A small, 0.188 mm wide, circular pharynx connects the anterior sucker with a long esophagus. The bifurcated caeca run parallel along either side of the body ending proximal to the caudal end of the fluke. The genital pore is ventral to the branched gut and has a small cirrus

pouch. Paired testes are located posterior to the acetabulum. The single ovary is positioned about the midline directly behind the testes. Brown, rather thick shelled ova measuring 0.037-0.040 mm x 0.025-0.027 mm are produced in the branched oviducts. The morphology of the fluke found in the gall bladder and bile ducts of cats in Hawaii is in agreement with this description (see Figure 37). Platynosomum semifuscum Looss and P. illiciens (M. Brn) are considered to be synonyms of P. concinnum (Rodrigues, 1963); however, careful comparison of the internal structures of these three flukes indicate they are separate species (Kossack, 1910).

The definitive host of Platynosomum concinnum is the domestic cat (Felis catus domesticus L.); however, the wild cat, Herpailurus y yaguarondi (Lac) (Barbosa et al., 1949) and the opossum, Didelphis marsupialis virginiana (Eckerlin et al., 1962) are also known to be infected in nature.

A few case reports and limited incidence studies show that a tropical climate is apparently required to complete the life cycle of P. concinnum as only cats in tropical areas become infected. Platynosomum concinnum has been reported from the following locations: Brazil (Barbosa et al., 1949), Puerto Rico (Garcia-Diaz, 1961), Malaysia (Rhode, 1966, Purvis, 1931), New Guinea (Talbot, 1968), South Florida (Eckerlin et al., 1962), Southern Texas (Powell, 1970), and Hawaii (Ash, 1962, Palumbo, 1974a). The prevalence of cat liver fluke infection in tropical areas appears to be high; however, most incidence reports are based on the examination of a limited number of animals.

A summary of the reported prevalence of cat liver fluke infection in various tropical areas is shown in Table 1.

Only one experimental study on P. concinnum has been made and that dealt mainly with the life cycle (Maldonado, 1945). In that study, Maldonado reported cats apparently became infected after killing and eating lizards. The infective stage, originally identified as a metacercaria, is found in the gall bladder and bile ducts of the intermediate host. In Hawaii the skink, Lygosoma metallicum, and the geckos, Hemidactylus frenatus and Gephyra mutilata, serve as sources of infective material (Sinclair, 1975). Experimentally, the infective stage has been shown to migrate up the common bile duct of mice and into the gall bladder (Maldonado, 1945). The route of migration in the cat is assumed to be the same. When the fluke reaches maturity in the biliary passages, it releases embryonated ova into the feces of the definitive host. Upon ingestion of the ova by the appropriate snail (Subulina octana and Eulata similaris (Ash, 1962) in Hawaii) the miracidium hatches, mother sporocysts are formed, followed by the development of daughter sporocysts. Cercariae produced within daughter sporocysts then emerge from the snail. The next step in the life cycle and how the lizard intermediate hosts become infected are not known. Studies on the biology of P. concinnum are not found in the literature other than those partly describing the life cycle of this parasite.

Obviously much of the fundamental biology and host-parasite relationship of P. concinnum has not been studied. Thus, the purpose of this dissertation was to answer some of the relevant questions

TABLE 1

REPORTED PREVALENCE OF *P. CONCINNUM* INFECTION

Location	Year	% Infection	No. Cats Surveyed	Reference
Brazil	1936	36	22	Torres & Pinto, 1936
Brazil	1963	55	40	Langenegger *et al.*
Puerto Rico	1961	89.5	290	Garcia-Diaz, 1961
Bahamas	1963	81.3	16	Leam *et al.*, 1963
Cuba	1946	100	NS	Kouri *et al.*, 1946
Malysia	1971	73.0	304	Retnasabapathy *et al.*, 1971
Florida	1962	31.6	9	Eckerlin *et al.*, 1962
Hawaii	1962	15	105	Ash, 1962
Hawaii	1974	41	203	Palumbo *et al.*, 1974

NS = Not specified.

concerning the host-parasite relationship between P. concinnum and the cat Felis catus. However, before an experimental study could be conducted, two important technical problems had to be resolved. The first required that all positively infected cats be correctly diagnosed through fecal examination. Although ova are passed in the feces of infected cats, the number of ova can be very small. Cats infected with P. concinnum are often undiagnosed by direct fecal smears. The formalin-ether technic, using one gram fecal samples collected on three consecutive days, and carefully scanning of all concentrated material microscopically, proved an effective method for the diagnosis of infected cats (Palumbo, 1974a).

The second technical problem was to find a means of experimentally infecting cats. Palumbo (1973) reported finding a parasite fluke stage within the bile ducts of the toad, Bufo marinus, morphologically similar to that found in the lizard intermediate hosts. In the current studies, when these infective stages were fed to kittens the parasites matured into adult P. concinnum. Thus, the infective stage found in the livers of toads (an apparent paratenic host) could be used as a source of infective material to produce platynosomiasis in cats.

A number of clinical, hematologic and serum biochemical, pathological, and immunological parameters were selected to monitor the host parasite interactions which occur between P. concinnum and the cat during the course of experimental infection. Some of the basic questions, and the rationale for the selection of certain parameters are discussed below.

1. What clinical signs and symptoms are associated with Platynosomum concinnum infection?

Various clinical symptoms have been noted based on the observations of a few case reports involving pet or stray cats. Symptoms reported for adult cats with naturally acquired platynosomiasis include weight loss, emaciation (Greve et al., 1966; Purvis, 1931), diarrhea (Jubb et al., 1970) vomiting (Retnasabapathy et al., 1971), and death (Cattcott, 1964). Individual animals with distended abdomens and jaundice have been reported (Powell, 1970). In general, most animals examined appeared healthy (Purvis, 1933). Symptoms noted and the number of flukes recovered at necropsy from each cat by various authors are summarized in Table 2. As is illustrated, the clinical symptoms may span the range from no symptoms to death; however, clinical observations under controlled conditions have not been made.

TABLE 2

SYMPTOMS AND FLUKE BURDEN REPORTED FOR NATURALLY INFECTED CATS

Number of Flukes/Organ				
Liver	Small Intestine	Other Organ	Clinical Observations	Reference
1 - 1000	200	Stomach (few)	Thin	Purvis, 1933
1 - 1000	-	-	Healthy	Garcia-Diaz, 1961
200 - 300	-	-	Emaciated	Cattcott, 1964
500	-	-	Death	Cattcott, 1964
+	-	Pancreas & Lungs	-	Purvis, 1933

Each cat was examined daily for clinical changes attributable to platynosomiasis, as well as noting any changes in the characteristic behavioral pattern of each cat.

2. What hematological, serum biochemical and serological changes are seen in the cat during P. concinnum infection?

Normal hematologic values for healthy cats have been established (Wilkins et al., 1975); however, alterations in felines parasitized with cat liver fluke infection have not been studied. Tests were selected to monitor liver function, anemia, proteinemia, leucocytosis, kidney involvement, and ion balance.

Assays of certain serum enzyme levels serve as useful diagnostic tools to evaluate the extent of liver damage. Such enzymes include glutamic oxaloacetic and glutamic pyruvic acid transaminases and alkaline phosphatase. These liver enzymes are elevated in other biliary trematode infections (Kearney et al., 1967). In addition, metabolic products such as bilirubin in serum and urine reflect alterations caused by parenchymal liver damage and biliary stasis.

Anemia is often seen in fluke infections. Digenetic flukes were originally considered to be blood feeders; however, this is not always the case. The anemia involved in fascioliasis is believed to be produced by leakage of blood from pathologically altered bile ducts (Holmes et al., 1968). Hypoalbuminemia may also result. Since blood has been reported in the bile of cats with liver fluke infection, a similar effect may be involved. The presence or absence of anemia was assayed using hematocrit (PCV), total erythrocyte count, total

hemoglobin, mean cell diameter, sedimentation rate, mean cell volume, mean cell hemoglobin content, and mean corpuscular hemoglobin content.

An increase in the number of eosinophils in circulating peripheral blood is characteristic of parasitic infection (Hudson, 1973). Leucocytosis often accompanies acute infections and occasionally persists in the chronic state (Schalm, 1962). Leucocyte changes were therefore closely followed throughout the experimental course of infection both to monitor the possibility of other underlying diseases as well as any changes due to liver fluke parasitism.

P. concinnum has been reported in organs other than the liver. One such site is the pancreas. Parasites in aberrant sites often produce considerable damage (Erasmus, 1972). Serum glucose helped assess pancreatic function.

Renal involvement in platynosomiasis has been reported by two investigators. In a case report, advanced hydronephrosis of the right kidney with enlargement and some degeneration of the left kidney was observed (Levine et al., 1957). Retnasabapathy (1971) found 56.2% of cat liver fluke positive animals had pale yellow kidneys with fatty infiltration; only 23% of uninfected cats had kidneys with this pathology. Serum blood urea nitrogen was measured to reveal any decrease in kidney function. Serum anions and cations were measured to judge the osmoregulatory capacity of the kidneys.

3. During the course of P. concinnum infection, what pathological events occur within the liver?

Several authors have conducted histopathologic studies on

naturally infected cats, usually with an undetermined fluke burden, and parasitized for an undetermined period (Greve et al., 1966; Robinson et al., 1962). They found that in advanced stages of the disease, the bile ducts show much the same pathology as in human clonorchiasis, with hyperplasia of the bile epithelium, cellular infiltration and fibrosis.

4. What is the developmental sequence of the parasite and how does the growth rate of the fluke relate to liver damage?

Cats used in this study were surgically biopsied prior to experimental infection to verify the normal hepatic histologic structure present. During the course of infection, cats were periodically sacrificed so that the progression of histopathological changes could be followed. Flukes, teased from bile ducts, were examined microscopically to establish their developmental stages.

Liver parenchymal and ductual cells have a normal turnover rate (Mac Donald, 1961). Following liver damage, cells must be repaired. Autoradiography using tritiated thymidine quantitates the number and types of cells which are actively synthesizing DNA and therefore indicates which of these cells are in the process of replacing damaged tissue. Autoradiographic studies were made during the course of experimental platynosomiasis.

5. Is the liver damage in platynosomiasis confined to the bile ducts areas or is there parenchymal involvement?

Parenchymal cells are reported to be histologically normal in fluke infested livers; however, changes in metabolic pathways have been

reported in several parasitic diseases (Simesen et al., 1973). Selected enzymes were studied in hepatic tissue throughout the course of P. concinnum infection.

6. Does a biochemical pathway exist which is responsible for the elimination and/or destruction of the flukes?

A mechanism for the elimination and/or destruction of digenetic trematodes has not been postulated. Methods devised for bacterial kill include 1) halogenation with iodine or chloride in the presence of hydrogen peroxide, peroxidase, and a hydrogen donor (Klebanoff, 1968), 2) conversion of the substrate of peroxidase to a toxic aldehyde or ketone (Strauss, 1970), and 3) the formation of free radical leading to membrane depolarization in the presence of hydrogen peroxide and ascorbic acid (Miller, 1969). All three killing mechanisms involve the production of hydrogen peroxide. Hydrogen peroxide has been shown to be deleterous in low concentrations to flukes (Chu, 1973). The oxidative enzymes which generate hydrogen peroxide are located in peroxisomes. These unit membrane bound vesicles originate from the endoplasmic reticulum and contain the enzyme catalase and other oxidative enzymes, usually d-amino acid oxidase (DAAO), l-α-hydroxy acid oxidase (OHAO), and uricase (UO). Liver parenchymal cells contain more peroxisomes than any other tissue (Hruban et al., 1969).

Eight enzymes were analyzed in liver tissue. These include the transaminases, glutamic oxaloacetic and pyruvic transaminases, which are located in the cell cytoplasm; peroxidase and catalase which are located in microbodies and the cell cytoplasm; and the peroxisomal enzymes,

DAAO, OHAO, and UO. Figure 1 shows schematically their metabolic relationships.

7. Does the presence of Platynosomum concinnum change the composition of feline bile?

The proteins in feline bile have not been studied; however, the electrophoretic evaluation of biliary proteins may provide useful information about the biology of P. concinnum. Neither the nutritional sources of the parasite nor the composition of its excretory products is known. A decrease in the number of bile proteins would indicate that a product in bile was being metabolized by the fluke as a nitrogen source. An increase in the number of electrophoretic bands would result from either a metabolic waste product produced by the parasite which could act immunologically as an exoantigen or by the leakage of an abnormal protein into the bile due to ductal or parenchymal damage.

8. During the course of infection does the antigenic expression of the parasite change?

Throughout the course of evolution, parasites and hosts have evolved together (Sprent, 1959). The result has been a trend toward reduction in antigenic disparity between the two members of the association. Parasites with surface antigens similar to the hosts would be less likely immunologically dislodged from the host. However, the complete abbrogation of antigenic disparity would not be beneficial since high parasitic populations would become established in immunologically nonresponsive hosts resulting in considerable pathologic damage (Damian, 1964). Millions of years were presumably required to

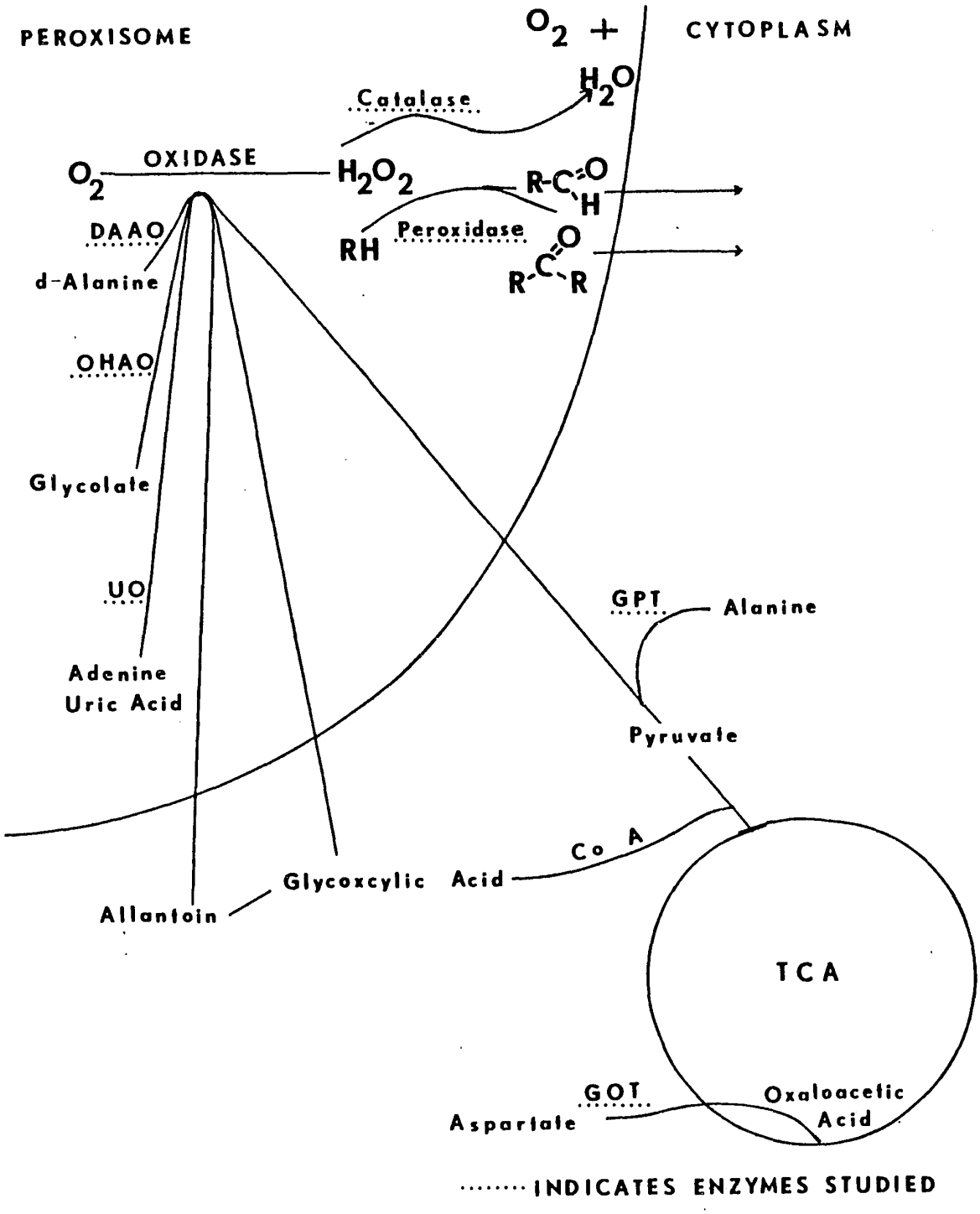


FIGURE 1

BIOCHEMICAL RELATIONSHIP OF PEROXISOMAL AND CYTOPLASMIC ENZYMES IN HEPATIC TISSUE

achieve antigenic similarity; however, Smithers (1967) has shown that schistosomes could absorb material from the serum of the host directly into their surfaces. The material binding to the surface of schistosomes is a glycoprotein with RBC antigens (Clegg et al., 1970).

The binding of specific immunoglobulins to parasite surfaces have been postulated for Nippostrongylus brasiliensis (Ogilvie, 1967). Plasmodium berghei (Jerusalem et al., 1971), Toxoplasma sp (Araujo et al., 1972), and Protostrongylus (Hudson et al., 1971). The antibody produced by the host in response to the parasite binds to the parasite surface, but are not sufficient to kill the parasite. Thus the "coated" parasite eventually becomes recognized as host material.

The ability of P. concinnum to bind host serum proteins and/or specific immunoglobulins to its surface was examined.

9. Does P. concinnum alter the immunological responsiveness of its host?

Current information indicates a parasite may have a second mechanism for dealing with the host's immune system--that of immunosuppressing its host, i.e. rendering the host unresponsive to certain antigens. Epidemiological evidence indicates an increased incidence of Burkitt lymphoma with malaria (Dalldorf et al., 1964), sarcomas in dogs with Spirocerca (Bailey, 1963), and cervical carcinoma in humans with Trichomoniasis (Bertini et al., 1970). Mice experimentally infected with Plasmodium berghei have reduced spontaneous lupu-like autoimmune disease (Greenwood et al., 1970); animals with Trichinella spiralis show enhanced carcinogenic effect to nickel sulfide (Kasprzak et al., 1971); mice infected T. spiralis displayed delayed homograft skin

rejections (Suet-Moldavsky et al., 1970), and mice with P. berghei infection had impaired responses to certain antigens but not to other antigens (Salaman, 1970).

The immune system of cats with P. concinnum was studied and compared with parasite free animals for signs of immunosuppression.

10. What is the role of cell mediated immunity in P. concinnum infections?

The role of cellular immunity in parasitic diseases is a fascinating but complex subject. Experimental studies have shown that lymphocytes, but not serum, can confer protection to challenge for certain parasites. For example, Cobra (1971) demonstrated that cattle which received sensitized lymphocytes (T cells) from syngenic donors had a reduced susceptibility to infection with Fasciola hepatica. Immune serum did not passively protect the same animals. Thus cell mediated immunity is apparently involved in protecting cattle against reinfection with Fasciola. The efficiency of humoral and cell mediated immunity for protecting cats against Platynosomum concinnum infection was studied.

Complete or partial answers to the above questions have provided new information concerning the host-parasite association of P. concinnum and its natural definitive host, Felis catus domesticus. The clinical symptoms and pathological changes discussed above for platynosimiasis are similar to those recorded for human clonorchiasis (Cheah et al., 1970). P. concinnum and C. sinesis are parallel parasites in that they both enter the definitive host by ingestion of an intermediate host, migrate up the common bile duct and establish

themselves in the biliary tract. Clonorchiasis is a major disease in the Orient and presently an animal model for experimental chemotherapeutic studies does not exist. It is hoped that the information presented in this dissertation may provide useful and relevant information which would be applicable for C. sinensis studies.

MATERIALS AND METHODS

I. Animal Husbandry Procedures

A special closed colony was established in Snyder Hall room 507 for this study. The cats were housed in a 12' x 20' room which receives twelve air changes per hour and has a constant temperature of 24°C. A twelve hour light/dark cycle beginning at 6 a.m. daily was maintained throughout the course of the study.

Two cats were housed in each cage. The cages were of galvanized steel and equipped with two carpeted wooden perches, a food and water dish and a large nalgene litter box.

The cages and food dishes were scrubbed daily with disinfectants (LpH-One Stroke Environ, Vestal Laboratories) or chlorox (Krystal Bleach, Oahu Chemical Works, Honolulu, Hawaii). The litter boxes were also changed daily. Wood shavings served as litter which were sterilized along with the litter boxes.

Each cat under eight pounds was fed 225 grams (8 oz.) of Purina Cat Chow (Ralston Purina Co., St. Louis, Mo.) every morning; cats over eight pounds received proportionally larger amounts of food. Each cat received 250 ml of fresh water daily. The diet was supplemented with vitamin tablets bimonthly (Pet Tabs, SeMed Pharmaceuticals, Bristol, Tenn.).

A log was maintained to record unusual behavioral patterns of the animals. Animals with signs of inappetence, lethargy, dehydration, cystitis, estrus, dermatitis, pneumonitis, diarrhea, vomiting or bite wounds were carefully examined. Treatment was administered as warranted

according to standard veterinary practices.

Weekly weight measurements were recorded for each cat and fecal samples were collected periodically (see Materials and Methods IV).

II. Methods for Selection and Conditioning of Felines

Not all live cats donated to the University were acceptable for use in a long term experimental study. Thus desirable cats had to be selected and conditioned prior to use. Cats were initially screened using the following criteria: they had to be 1) adult cats at least 4 1/2 pounds in weight, 2) animals in good physical condition (free from emaciation, thinness, dehydration, diarrhea and other obvious diseases such as bone tumors and lymphomas), 3) females that were not pregnant, 4) felines that were intact--neither neutered nor spayed, 5) cats that were free of skin lesions (such as various dermatitis, ringworm, mange, bite wound abscesses, extensive ear mite infections or any undefined hypersensitivities). A fecal sample was collected. Cats passing ova of *P. concinnum*, *Aelurostrongylus abstrusus*, or *Anafilaroides rostratus* were eliminated because treatment for cat liver fluke and lungworm infections is not available. A blood sample was then collected. Cats displaying behavioral problems during blood sample collection were also eliminated. Animals with abnormally elevated blood urea nitrogen (BUN) or transaminase enzyme levels were eliminated along with cats with feline infectious anemia (*Hemobartonella felis*). Seventy-five percent of the cats examined were eliminated following this screening procedure.

Of the twenty-five percent of the cats selected, two were Siamese, one was Russian Blue and twenty-one were domestic short-hair.

All cats were treated for intestinal parasites with either DNP (2, 6-diiodo-4-nitrophenil, American Cyanamid Co., Princeton, N. J.) injected subcutaneously at a dose of 0.1 ml per pound body weight or omnizole (Thiabendazole, Merck Chemical Division, Rahway, N. J.) administered orally at a dose of 2 ml per cat. Treatment was repeated 21 days later. Treatment was again repeated if needed at three week intervals until all cats were fecal negative for all detectable intestinal parasites. Each cat was vaccinated for feline panleucopenia (feline enteritis-distemper) using killed virus of tissue culture origin (Burns-Biotec Laboratories, Inc.). A booster immunization was given 7--10 months later to all cats.

During the conditioning period each cat contracted viral pneumonitis. The outbreak lasted approximately 12 weeks. Animals found sneezing or with signs of pneumonitis received a regime of 0.2 ml Bicillin/kg I.M. (Sterile Benzathine penicillin G and Procaine penicillin G in aqueous suspension, Wyeth Laboratories, Inc., Phil., Pa.) weekly and one ml vitamin B complex I.M. (Trico Pharmaceutical Co., Oregon City, California), every five days for approximately three weeks. Three months after the initial outbreak most of the cats had completely recovered.

III. Procedure for Infecting Cats with Platynosomiasis

Bufo marinus livers were used as a source of infective materials in all studies discussed in this dissertation. Over 850 toads were captured at various times of the year from Manoa Valley (44.1%), Wahiawa (26.5%), Aina Haina (25.9%), and Kailua (3.6%).

Previous studies showed that approximately 65% of the toads from these areas contained the infective stage (metacercaria), Plate 1, of P. concinnum within their livers (Sinclair, 1975).

In general, the toads were anesthesized with chloroform and the livers excised. The gall bladders were immediately removed from each liver as toad bile was believed to make cats vomit. The livers were then washed free of blood with running tap water and gently blotted with paper towels. Each liver was then cut into 5 mm cubes with scissors. All liver pieces were pooled and the total weight determined. Aliquots of the polled liver pieces were weighed and presented to the conditioned cats within one hour after killing the toads. The cats usually consumed all the liver within an hour. The infective dose was thus estimated from an aliquot of the polled liver pieces. The pieces of liver were further minced in saline and the number of infective stages present were determined using a dissection scope.

Two doses were administered. Group A (8 cats) served as uninfected controls; Group B (13 cats) received a low dose (less than 400 infective stages); Group C received a high dose (approximately 2000 infective stages). Table 3 shows the dates of infection and the infective dose given to each cat in the study. Two cats (#30 and 31) were infected as kittens with an undetermined dose of infective stages. These cats comprise Group D and are being held to establish the length of P. concinnum infection. Three naturally infected cats (#32 and 34) were fed a secondary challenge dose. These are placed together in Group E.

TABLE 3

THE INFECTIVE DOSE AND BLEEDING SCHEDULE
FOR EXPERIMENTALLY INFECTED CATS

Group	Cat No.	Sex	Biopsied +/-	No. Infective Stages [a]	Sacrificed	Bleeding Schedule [b]
A-Control						
	1	M	+	0	-	E
	2	M	+	0	-	0
	3	M	+	0	2 weeks	-
	4	M	+	0	-1 week	-
	5	F	+	0	-	0
	6	F	-	0	-	0
	7	F	+	0	-	E
	8	F	-	0	-	E
B-Low Dose						
	9	M	-	328	1 week	0
	10	M	-	240	3 weeks	0
	11	M	-	328	3.5 weeks	E
	12	M	+	240	6 weeks	E
	13	M	+	240	9 months	E
	14	M	+	240	-	0
	15	M	+	240	-	0
	16	M	-	240	-	E
	17	F	-	328	2 weeks	E
	18	F	+	240	9 months	0
	19	F	+	240	-	0
	20	F	-	350	-	E
	21	F	-	189	-	E
C-High Dose						
	22	M	+	1978	9 months	E
	23	M	+	1978	9 months	E
	24	M	+	1978	-	0
	25	M	+	1978	-	0
	26	F	-	1953	4 months	E
	27	F	-	1978	6 months	0
	28	F	+	1978	-	E
	29	F	+	1978	-	0
D-Long Term						
	30	M	-	ND	-	E/0
	31	F	-	ND	-	E/0
E-Secondary Challenge						
	32	M	-	477	3 weeks	-
	33	F	-	517	8 weeks	-
	34	F	-	712	8 weeks	-

a. ND = not determined.
b. E = even numbered weeks.

IV. Procedure for Fecal Examination

A modification of the standard formalin-ether technic (Ritchie, 1952) was used (Palumbo, 1974a). The fecal material was suspended in 10 ml of normal saline, passed through a fine wire mesh filter (325 holes/cm^2), and centrifuged in a clinical centrifuge. The supernatant was discarded and the pellet resuspended in 7 ml 10% buffered formalin. Fifteen minutes later 3 ml cold ether was layered on the top, the tubes were shaken vigorously and centrifuged. The supernatant and lipid soluble layer were discarded and the remaining pellet was resuspended in two drops of saline. The pellet containing concentrated ova was placed under two 22 mm^2 coverslips. Both slides were scanned completely and the number of ova counted.

For experimental animals, fecal samples were collected weekly the first seven weeks of infection. At the end of this time, daily samples were collected until ova were being passed. Subsequent samples of feces were collected at monthly intervals. Approximately 1 gram of feces was collected and studied by the formalin-ether technic until the cat started to pass ova. Once an animal was deemed positive, 1 gm of fecal material was weighed out, concentrated by the formalin-ether technic and the number of ova/gm of feces recovered by the formalin-ether technic.

V. Procedure for Hematology and Serum Biochemistry Studies

A. Blood Collection Protocol: The cats were manually restrained by an animal caretaker while being bled. Blood was collected from the cephalic vein by placing a tourniquet around the upper forearm. The

vein was palpated and two to three ml heparinized whole blood was collected in a disposable syringe using a 23 gauge, 3/4 inch needle. The venipuncture sites were alternated bimonthly when possible. Very little sclerotization was evident even after nine months of repeated venipuncture.

Blood was usually analyzed immediately after collection but on rare occasions it was refrigerated overnight before use. The two to three ml heparinized whole blood was sufficient to conduct all of the tests listed below.

Baseline hematological values were determined for each animal before and after liver biopsy. After infection cats in Groups A, B, and C were bled bimonthly for the first six months following experimental infection and monthly thereafter. Half of the cats from each group were bled on odd numbered weeks and half on even numbered weeks. Table 3 shows the bleeding schedule on each animal. Group D cats were bled periodically.

B. Leukocyte Count (WBC/cmm.): The heparinized whole blood collected above was diluted 1:20 with 2% acetic acid in a white blood cell diluting pipet and mixed on a mechanical shaker (Burton Model No. 1406, Pipette Shaker, Burton Manufacturing Company, Santa Monica, Cal.). After expelling the first six drops from the pipet, a hemacytometer with an improved Neubauer ruling (Spencer Bright Line AO No. 1475 Hemacytometer, Scientific Products Inc., McGaw Park, Illinois) was filled and the cells allowed to settle for a few minutes. Under high dry magnification (x430) the white blood cells in the four large, outer corner squares were counted and the total number of WBC per cubic

millimeter was then calculated.

C. Erythrocyte Count (RBC/cmm.): Heparinized whole blood was diluted 1:200 with 0.85% saline in a red blood cell diluting pipet and mixed on a mechanical shaker. After discarding the first six drops, the hemacytometer was filled. After allowing the cells to settle, the total number of red blood cells were counted in five of the small center squares under high dry magnification (x430). The total number of erythrocytes per cubic millimeter was then calculated.

D. Mean Erythrocyte Diameter (microns): An aliquot of heparinized whole blood, diluted for the erythrocyte count was also used to determine the mean erythrocyte diameter of these cells using the principle of light diffraction. After 5 minutes to allow the cells to settle on an erthrocytometer slide, it was placed in a Haden-Hausser instrument (Haden-Hausser Erthrocytometer, Clay-Adams Co., N.Y., N.Y.) and the mean size of the erythrocytes of the film in microns was read directly from the colored ring spectrum formed by manipulating the calibration wheel.

E. Packed Cell Volume (PCV %): Microhematocrit capillary tubes (75 x 1.5 mm) were filled with heparinized blood and centrifuged in a hematocrit centrifuge (National Micro-capillary Centrifuge model M-B, International Equipment Co., Needham Hts., Mass.) for 5 minutes at 1500 rpm. The packed cell volume of the sample was calculated using a reader (Critocap Micro-Hematocrit Reader, Biological Research, Inc. St. Louis, Mo.).

F. Erythrocyte Sedimentation Rate (mm/hr.): The erythrocyte sedimentation rate and the corrected sedimentation rate followed

standard veterinary methods described for dogs (Schalm, 1965) and mongooses (Palumbo, 1974b). The erythrocyte sedimentation rate was measured in mm every 15 minutes for one hour in the hematocrit tube prepared above.

G. Glucose (mg/100 ml): A drop of heparinized whole blood was placed on commercially available reagent strips containing glucose oxidase, peroxidase and a chromagen indicator (Dextrostix, Ames Co., Elkhart, Ind.). Exactly sixty seconds later the blood was washed off and the color produced compared to standards.

H. Hemoglobin (Hb gm/100 ml): Commercially available reagents were used (Hycel Cyanmethemoglobin Dry Pack Reagent #116E and Hycel Cyanmethemoglobin Certified Standard #127, Hycel, Inc., Houston, Texas); 0.02 ml of heparinized whole blood was added to 5 ml of cyanmethemoglobin reagent using a Sahli pipet and the optical density was measured photometrically at 540 mm (Coleman Junior II Spectrophotometer, Coleman Instruments, Maywood, Illinois). The optical density was converted to gm/100 ml by the use of a standard curve.

I. Blood Smear Differential: A standard thin film blood smear was prepared on a clean 1" x 3" glass slide, air dried, and fixed in absolute alcohol. The slide was stained for 30 minutes with Giemsa stain diluted 1:50 with water (Harleco Giemsa Stain #620, Harleco, Inc., Gibbstorm, N.J.). The slide was then rinsed with water and air dried.

Initial study of the blood smear was made under high dry magnification (x430) to verify a normal distribution of cells. The differential leucocyte count was conducted with oil immersion (x970) using the so-called battlement scanning method (MacGregor et al., 1940).

At least 100 leukocytes were counted and the percent distribution recorded with a Marbel blood-cell calculator (The Marbel Blood-cell Calculator Co., Chicago, Ill.).

J. Platelet Count: The total number of platelets/cmm was estimated from the Giemsa stained slide discussed above. The count was considered to be "adequate" when the number of platelets/100 WBC multiplied by the total number of WBC exceeded 1.5×10^5 per cmm.

K. Total Protein (gm/100 ml): A total solids or TS meter was used (National Instrument Co., Wellmore, Md.). This refractometer was designed specifically for the accurate measurement of total solids in urine, serum, and plasma (Rubinis, 1957, Barry et al., 1960). The refractive index reflects the amount of total solids present with an accuracy of $\pm$0.1 gm percent. The total protein scale has been compensated for certain common nonprotein constitutents of serum or urine.

A drop of serum, plasma, or urine was placed on the prism of the refractometer and the total protein and refractive index scales were read through the eye piece.

L. Albumin (gm/100 ml): Albumin concentration was determined photometrically using commercially available reagents (Protein A/G Kit, OmniTec, Inc., Santa Monica, Cal.). Serum or plasma (0.1 ml) was added to 1.9 ml of 27.8% sodium sulfate-sodium sulfite solution and mixed well. The addition of 1 ml anhydrous ether precipitated the globulins which were removed by centrifuging the mixture at 1500 rpm for 5 minutes. A one ml aliquot of the solution containing the albumin was added to two ml Biuret reagent (stabilized 0.15% copper sulfate in

potassium tartrate, sodium citrate and sodium hydroxide). After 10 minutes to allow for full color development, the optical density was measured at 550 nm. The concentration of albumins was determined by comparison with a standard curve.

M. Globulin Determination (gm/100 ml): Since the total protein concentration in serum or plasma was determined refractometrically and the albumin was determined with the the method described above, the concentration of globulins was determined by calculation, as was the A/G ratio.

N. Formolgel Test--Test for Hyperglobinemia: A 0.2 ml sample of serum or plasma was added to 0.05 ml of neutral buffered formalin and incubated at room temperature overnight. Positive samples formed a gel within 24 hours.

O. Icterus Index: The icterus index (an index of jaundice) was estimated by comparing the color of the serum of plasma with a series of color standards prepared by diluting potassium dichromate with distilled water (Ham, 1958).

P. Bilirubin: Reagents for bilirubin analysis are available commercially (Bilirubin Test Set #290A, Hycel, Inc., Houston, Texas). The procedure is based on the method of Malloy and Evelyn (1937). A 0.05 ml sample of serum or plasma was added to 1.0 ml of solution A (1% sulfanilic acid in 0.22 M HCl) and then mixed with 1.0 ml of solution B (0.1% sodium nitrite and 20% sucrose W/V). At a moderate pH bilirubin formed a typical purple azo-bilirubin color. Methyl alcohol was used as an accelerator.

Q. Blood Urea Nitrogen (BUN: mg/100 ml): Chromatographic reagent

strips, available commercially, were used to determine blood urea nitrogen (Urograph, Warner-Lambert Co., Morris Plains, N.J.). These reagent strips utilize essentially the chemical reactions of the Conway microdiffusion method (1958). The strips are placed in 0.2 ml of serum or plasma. During the 30 minute incubation period, digestion of the urea in the sample by buffered urease produced ammonia which was released as a gas by K_2CO_3. The gas was then quantitated chromatographically with tartatic acid in the presence of bromcresol green indicator so that the reaction of gas migration could be compared to the standards.

R. Alkaline Phosphatase (Bodanski Units): The method of Klein et al. (1960) was used for the determination of serum alkaline phosphate levels (Phosphatabs--alkaline quantitative, Warner-Lambert Co., Morris Plains, N.J.). In this procedure 0.05 ml of serum was added to 0.125 ml of buffered substrate consisting of phenolphthalein diphosphate in Tris buffer, pH 9.6. After 30 minutes incubation at 37°C the reaction was stopped, and the phenolphthalein indicator developed by addition of 1.25 ml of sodium hydroxide. The optical density was measured at 550 nm and compared to a standard curve.

S. Serum Glutamine Pyruvic Transaminase (SGPT: SF Units): Commercially available reagents (Kit #505P, Sigma Chemical Co., St. Louis, Mo.) were used in the photometric determination of SGPT levels according to the Sigma modification of the method of Reitman and Frankel (1957). In this procedure, 0.05 ml serum of plasma was added to 0.25 ml of substrate (buffered alanine, pH 7.5) for 30 minutes at 37°C. Then 0.25 ml dinitrophenyl hydrazine in HCl was added and the

mixture kept at 25°C for 20 minutes. Next 2.5 ml of 0.40N sodium hydroxide was added and the mixture read in the Coleman Spectrophotometer at 505 nm after a 10 minute incubation period at room temperature. The optical density was converted to SF units by using a standard curve.

T. Serum Glutamic Oxalacetic Transaminase (SGOT: SF Units): The colorimetric determination of SGOT levels in serum and plasma (Sigma Kit #505). In the Sigma method, 0.05 ml of serum or plasma was incubated with 0.25 ml of pH 7.5 buffered aspartate -α-ketoglutarate substrate for 60 minutes at 37°C. Dinitrophenyl hydrazine in HCl (0.25 ml) was added and the mixture incubated at room temperature for 20 minutes. Then 2.5 ml of 0.40 N sodium hydroxide was added and the optical density determined at 505 nm after a 10 minute incubation period at room temperature. A standard curve was used to convert the OD to SF units.

U. Chloride and Calcium Determinations: The principle of the chloride determination was that of Schales and Schales (1941). A 0.05 ml sample of serum or plasma was added to 3 drops 0.1 M nitric acid and 1 drop s-diphenylcarbazone in ethanol in a titration cup (Oxford 3 ml titration cups) and immediately titrated with 0.01 N mercuric nitrate. Chloride ion concentration was determined by comparison with a standard of 100 mEq/l.

The principle of the calcium determination is a modification of the original titrimetric method of Bett and Fraser (1958). In this method 0.05 ml of serum or plasma is added to 3 drops of deionized water in a titration cup. One drop of 6.25 M potassium hydroxide and

one drop of 25.0 mg% calcein are added immediately before titration with 2.5×10^{-4}M EDTA.

Oxford chloride reagent #630 and calcium reagent #631 were used to determine serum chloride and calcium by titration in Oxford 3 ml titration cups using an Oxford Sampler Micropipetting System #805 (Oxford Reagents and Equipment, Foster City, Cal.).

V. Inorganic Phosphorus (mg/100 ml): In this method 1.5 ml of a commercially available reagent (Hycel Phosphorus Reagent, #198A, Hycel, Inc., Houston, Texas) was added to 0.5 ml serum or plasma. The phosphorus reagent contained 0.19% ammonium molybdate, 0.39% ferrous ammonium sulfate, 5.32% sulfuric acid and 1.45% polyoxyethylene ether. After incubation at room temperature for 30 minutes the optical density of the reaction mixture was measured at 650 nm. A phosphorous standard (Hycel phosphorus standard #199) equivalent to 8 mg/100 ml of serum phosphorous was used to prepare a standard curve.

VI. Methods for Urine Analysis

Urine samples were analyzed immediately after collection. The total volume, transparency, color, odor, refractive index, and specific gravity (see Materials and Methods, V.--K.) were noted. Commercial reagent strips were used for biochemical urinalysis (Multistix, Ames Co., Elkhart, Ind.). These plastic strips had seven separate reagent zones for determination of pH, protein, glucose, ketones, bilirubin, occult blood and urobilirubin. A drop of urine was placed on each area, washed off with water at a specified time and compared to a color standard.

A. pH: This zone contains a double indicator of methyl red and bromethymol blue.

B. Protein: This test is based on the protein error of indicators principle since at a constant citrate buffered pH any color which develops with the included tetrabromphenol blue indicator is due to the presence of protein.

C. Glucose Test: This zone utilized a double sequence enzyme reaction. The reaction first utilized the enzyme glucose oxidase to catalyze the formation of gluconic acid and hydrogen peroxide for the oxidation of glucose. In turn, a second enzyme, peroxidase, catalyzed the further oxidation reaction of hydrogen peroxide with the chromogen orthotolidine, to form blue oxidized orthotolidine.

D. Ketone Test: The reaction was based on the purple color developed with acetoacetic acid or acetone and nitroprusside, which was stabilized in a dry reagent containing glycine as the nitrogen source and a strongly alkaline buffer.

E. Bilirubin Test: The reaction was based on the coupling of bilirubin with diazotized dichloroaniline in a strong acid medium. The color developed gave tan to brown shades for positive bilirubin results.

F. Occult Blood Test: The detection of occult blood was based on the peroxidase-like activity of hemoglobin which catalyses the reaction of peroxidase and the chromogen orthotolidine, to form blue oxidized orthotolidine.

G. Urobilinogen Test: This zone utilized the Ehrlich reaction modified as a dry reagent, based on para-dimethylaminobenzaldehyde, which reacts with urobilinogen in strongly acid medium to form a red

color.

H. The urine not used in the above tests was centrifuged and the sediment was examined microscopically for 1) epithelial cells, 2) leucocytes, 3) erythrocytes, 4) bacteria, 5) casts, and 6) crystals.

VII. Procedure for Surgical Biopsies of Feline Hepatic Tissue

Liver biopsies were performed on 18 cats to establish that the livers were morphologically and histologically normal prior to infection. Surgical biopsies were initiated in March 1974 but an outbreak of viral pneumonitis in the colony required subsequent biopsies to be delayed until July 1974.

The same general procedure was used throughout. The felines were fasted 24 hours prior to surgery. Anesthesia was induced by a rapid venous injection of one-half the calculated total dose of a mixture of 1/3 nembutal sodium (Sodium pentobarbital injection, Abbott Laboratories, North Chicago, Ill.) and 2/3 pentothal (Sodium thiopental injection, Abbott Laboratories, North Chicago, Ill.). The remainder of the anesthesia was titrated while monitoring the palpebral, pupillary, cutaneous, and pedal reflexes. The abdomen was shaved, washed with surgical soap, and sterilized with alcohol. Sterile surgical drapes delimited the operating area.

A midline incision, approximately 3--6 cm long, was made with a No. 10 or 11 scapel blade. The peritoneal cavity was then entered using scissors. The posterior portion of the liver (usually right lobe) was revealed, the surface was examined, and the gross pathology recorded. A small portion of the posterior-most lobe was removed. The biopsied section was approximately 10--15 mm long and 3 mm wide.

Hemorrhaging was not a major problem. Adipose tissue lining the abdominal incision was teased away. The peritoneal lining, muscle and fascia were closed approximately 6-8 single stitches using 4-0 multifilament stainless steel wire ("Cabelwire" No. 2444, size 4-0, United Surgical Supplies Co., Port Chester, N.Y.). The skin was closed with 5-7 individual or mattress sutures. An injection of Bicillin I.M. was given following surgery. All animals recovered from anesthesia one hour after surgery.

The biopsied material was divided into three parts. One was fixed in 10% buffered formalin for histological sections, the second was incubated in Ringers saline with tritiated thymidine for regeneration studies and the third was frozen for enzyme analysis (see Materials and Methods, IX).

VIII. Necropsy Protocol

Cats were anesthetized with nembutal. While in a deep plane of anesthesia, 50 ml blood was obtained via cardiac puncture. The animal was then sacrificed with an anesthetic overdose.

Each cat was examined externally for signs of disease.

A mid-abdominal incision was then made. All internal organs were checked for location, number, size, and gross pathology. Special notice was paid to the size of spleen, and the size and characteristics of mesenteric and hepatic lymph nodes. Kidneys were removed, cross sectioned, and the capsule peeled from the organ. A urine sample was taken directly from the urinary bladder with a needle and syringe. The liver and common bile ducts were then removed and stored for further examination. A fecal sample was collected.

Tissues of interest (liver, kidney, spleen, hepatic and mesenteric lymph nodes) were fixed in 10% buffered formalin. A bile sample was removed from the gall bladder and examined microscopically for the presence of fluke ova. The bile was then stored. A portion of the liver was frozen for enzyme analysis.

The lobes of the liver were separated, weighed and the flukes teased from the minced liver. A dissection scope was used to locate the smaller flukes. The liver was examined until all flukes present were located. These flukes were placed in normal saline for 12 hours and then their individual length and width were measured and their individual development stages was noted.

Serum was collected and frozen. The number of ova/gm of feces was determined as previously described. A standard urine analysis was performed (see Materials and Methods, VI).

IX. Procedure for the Analysis of Liver Enzymes

A. Collection of Materials: One hundred and fifty-five cats were obtained either live as donations to the University of Hawaii School of Medicine or as dead animals immediately after euthanasia at the Humane Society. Animals donated to the University were unwanted pets; while those from the Humane Society had been either pets or strays. The cats were euthanized with nembutal (UH) or by decompression (HS). Immediately after death a heparinized whole blood sample was obtained by cardiac puncture. Each cat was then examined for external signs of disease (i.e., poor coat condition, signs of tumors, trauma or wounds, presence of nasal or ocular secretions, unthriftiness, diarrhea,

anemic pallor, or thinness). The sex and whether castrated or spayed, pregnant or lactating, as well as the approximate age of each feline was recorded.

A rapid necropsy, as previously described was performed (see Materials and Methods, VIII). The entire liver with the gall bladder and common hepatic duct was removed. A fecal sample was collected directly from the most distal portion of the colon. A small portion of the posterior right lobe was immediately removed and frozen in a sealed container for subsequent enzyme analysis. Bile was collected with a syringe and needle directly from the gall bladder, examined microscopically for the presence of fluke ova, and frozen in screw capped vials for future study. The liver was then divided into its four major lobes and each lobe was weighed to the nearest half gram. The liver was then minced into 0.5 cm pieces, liver flukes were teased from the bile ducts and counted. Isolated flukes were either washed once in saline and frozen to be used as antigenic material for certain immunologic studies or fixed in 10% buffered formalin for histopathologic study.

Serum or plasma was collected from the blood sample for transaminase level evaluation, as well as certain immunologic studies.

Fecal material was examined for the presence of the following parasite ova: Ancyclostoma caninum (hookworm); Aelurostrongylus abstrusus, Anafilaroided rostratus (lungworms); Toxocara canis, Toxocara cati, (roundworms); Isospora felis, Isospora rivolta, Isospora bigemina, Toxoplasma gondii (coccidia), (Ash, 1962, Alicata, 1964).

B. Preparation of Liver Extract for Enzyme Analysis: Liver samples were stored at -20°C until analyzed. A 10% extract of the frozen liver was prepared with a tissue homogenizer using distilled water as diluent. The extract was centrifuged at 1500 rpm for 5 minutes in the cold, the supernatant decanted and analyzed for enzyme activity.

C. Method for Analyzing Hepatic Transaminases (SGOT and SGPT): A 1:100 dilution of the above 10% extract was prepared. The same methodology as for serum was then used for these enzymes.

D. Method for Analyzing Catalase: The catalytic activity was measured according to a modification of Beers and Sizer (1952) which has been described in detail previously (Hokama et al., 1974). A one ml aliquot of a 1:100 dilution of the initial liver extract was added to 2 ml of a 5% solution of hydrogen peroxide. The rate of H_2O_2 disappearance was monitored spectrophotometrically at 240 nm. Values are expressed as K/N where K is the first order reaction rate and N is mg liver nitrogen. Total protein nitrogen was measured at 280 nm.

E. Method for Analyzing Peroxisomal Enzymes: The DAAO, OHAO, UO and peroxidase analyses were determined by a fluorometric procedure (Hokama et al., 1972) which was based on a procedure devised previously for glucose analysis (Guilbault et al., 1968). In brief, these oxidases react with their specific substrates to produce hydrogen peroxide which in the presence of horseradish peroxidase (HRP) converts homovanillic acid (HVA) to a fluorescent complex detectable with a fluorometer. The reaction rates produced by a 10% liver extract gave values corresponding to first order reaction rates.

Thus, the rate of fluorescent HVA-complex formed corresponded directly to increasing amounts of purified standard enzyme preparations.

1. DAAO: A portion of the extract (0.1 ml) was added to 0.5 ml of 40mM d-alanine, 0.2 ml HVA (2.5 mg/ml), 0.1 ml HRP (100 u/ml) and 1.4 ml Tris-HCl buffer pH 8.5, 0.1 M. The mixture was incubated 10 minutes at room temperature and the rate of HVA-complex formation was measured in a MPF-3-Perkin-Elmer Fluorescence Spectrophotometer at a sensitivity of 1.0 and the reaction rate was measured with a Hitachi continuous recorder.

2. UO: A portion of the extract (0.1 ml) was added to 0.2 ml HVA, 0.1 ml HRP, and 1.9 ml 42uM sodium urate. The reaction rate was measured as for DAAO.

3. OHAO: A portion of the extract (0.1 ml) was mixed with 0.2 ml HVA, 0.1 ml HRP, 0.5 ml 25mM sodium glycolate and 1.4 sodium pyrophosphate-Trition pH 7.4 buffer and the reaction rate was determined as above for DAAO.

4. Peroxidase: A portion of the liver extract (0.1 ml), 0.2 ml HVA, 0.1 ml 1.0% sodium azide and 1.8 ml Tris-HCl buffer (pH 8.5, 0.1M) were mixed and incubated at room temperature for 2 minutes. Then 0.1 ml of 0.01 M hydrogen peroxide was then added and the reaction rate was measured.

The age of the cat, its sex, the enzyme levels, and the parasite burdens were entered onto computer cards. The data was analyzed on the University of Hawaii Campus Center's IBM 370/165 with the SPSS and BMD packages (Harris, 1975).

X. Procedure for Electrophoresis of Bile Proteins

Twenty bile samples were used. Ten positive and ten negative samples were electrophoretically analyzed on cellulose polyacetate strips and on polyacrylamide gels.

Sephraphore III cellulose polyacetate electrophoresis strips (Gelman Instrument Co., Ann Arbor, Mich.) were soaked overnight in Tris-barbito-sodium barbitol buffer, pH 8.8, 0.06M. Bile (2-3 lambda) was applied to each strip and electrophoresed at 1.5 ma per strip for 45 minutes using the same buffer. A Gelman power source was used. The strips were stained with Ponceau S stain for 10 minutes, destained with three changes of 5% acetic acid, dehydrated with absolute alcohol and mounted on 1" x 5" glass slides. When dry, the transparent strips presented red protein bands. The strips were scanned with a Densicord-recording electrophoresis densitometer (Photovolt Corp., N.Y., N.Y.).

An upper and lower gel was prepared for polyacrylamide disc electrophoresis. The procedure of Seto et al. (1964) was generally followed. The lower gel was prepared by combining tris-(hydroxy methyl) aminoethane (8.9 gm), N,N,N,N,-tetramethylethylenediamine (0.125 ml), acrylamide monomer (15.0 gm), N,N-methylenebiacrylamide, monomer (0.4 gm), 1M HCl (12.0 ml), potassium ferricyanide-0.15% solution in water (0.5 ml), distilled water (q.s. 100 ml). To this solution an equal volume of ammonium persulphate (35 mg/25 ml water) was added as a catalyst immediately prior to use. The above solution was poured into 10 x 60 mm glass tubes and allowed to polymerize under U.V. treatment.

A small uppergel was added. It was composed of 1M phosphoric

acid (6.5 ml); tris-(hydroxymethyl) aminomethane (1.5 gm), acrylamide, monomer (5.0 gm); N, N-methylenebiacrylamide, monomer (1.25 gm); riboflavin (1.0 mg); and distilled water (q.s. 100 ml).

Bile (20 lambda) was layered on the surface of the upper gel. The tubes were electrophoresed at 5 milliamperes/tube for 60 minutes in glycine-tris buffer. The gels were stained in amidoschwartz dye for 60 minutes, destained with 7.5% acetic acid and scanned with the Densicord.

XI. Procedure for Preparation of Tissues for Histological Studies

Tissues were stored in 10% buffered formalin until used. Blocks measuring approximately 1 cm square by 0.2--0.5 cm thick were embedded in paraplast (melting point 56°C). One of the following two schedules were used (Pearse, 1960). Schedule A: 6--12 hours 95% ethanol (3 changes), 6--12 hours 199% ethanol (2 changes), 6--12 hours methyl-benzoate celloidin (2 changes), 15 minutes benzene (2 changes), 30--60 minutes 1:1 benzene to paraplast, and 6--12 hours paraplast (2 changes). Schedule B: One to hours each in 50% ethanol, 70% ethanol, 95% ethanol, 100% ethanol (2 changes), 1 hour xylene, 1 hour 1:1 xylene : paraplast, and 1 hour paraplast (2 changes). Embedded tissues were sectioned 6--10u in thickness with a microtome and mounted on alcohol cleaned glass slides. The paraplast was removed and the sections were stained according to the following steps: xylene--5 minutes (2 changes), 100% ethanol--5 minutes (2 changes), 95% ethanol (1 minute), 70% ethanol--1 minute, Harris hematoxylin--4 minutes, running water--5 minutes, 70% acid alcohol--1 minute, 70% alkaline ethanol--2 minutes, 95% ethanol--1 minute, alcohol-eosin 0.5--

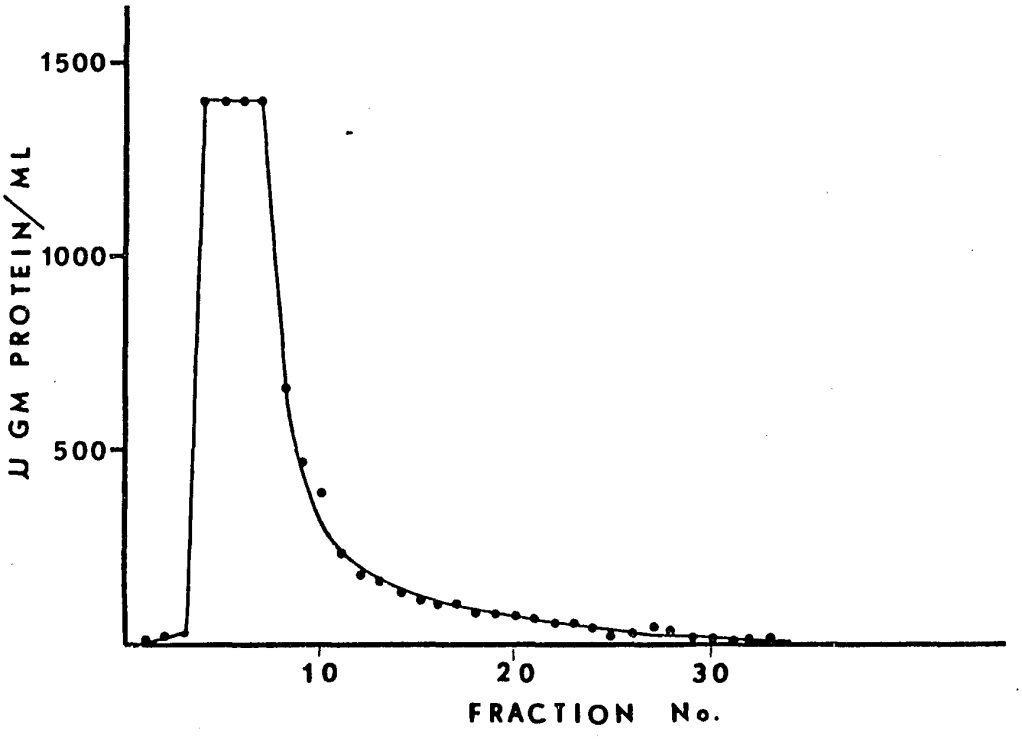
1 minute, 95% ethanol--1 minute (2 changes), 100% ethanol--5 minutes (2 changes), xylene--5 minutes (2 changes). The sections were mounted in Permount.

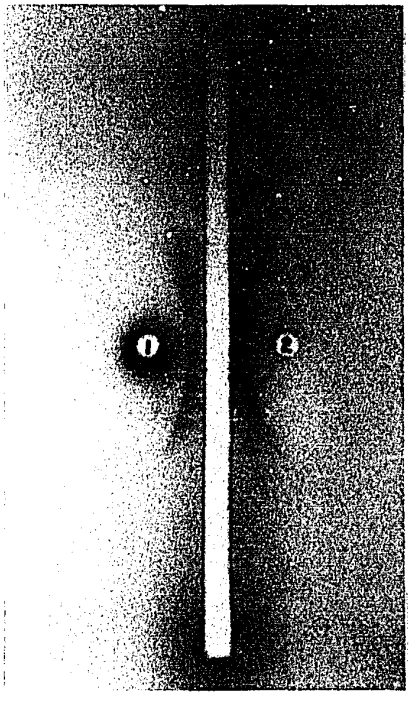
XII. Protocol for Autoradiography-Regeneration Capacity of Hepatic Tissue

The procedure of Leevy (1963) was followed. A3--5 mm cube of hepatic tissue was obtained from biopsy specimens or at necropsy. The liver material was washed in mammalian Ringers and incubated in a screw cap tube containing 3 ml mammalian Ringers, 50 μCi methyl tritiated thymidine (New England Nuclear, Boston, Mass.), and 75 units hyaluronidase at 37°C. After 90 minutes the tissue was removed and fixed in 10% buffered formalin. The tissue was then embedded in paraplast (Materials and Methods, XI) 5--8 u sections prepared, and placed on 4 slides. The slides were then dipped three times in NTB-2 Kodak emmulsion, allowed to dry and exposed in a light-tight box at 4°C for 3, 5, 8, and 12 weeks. The autoradiographs were developed with Kodak D-19 developer for 2--5 minutes, fixed for 4 minutes in Kodak fixer diluted 1:1 with water and washed in cold running tap water for 30 minutes. The slides were stained with Harris hematoxylin, counter-stained with eosin and mounted in permount as described previously. Estimates on the numbers of nuclei exhibiting DNA syntheses were made. The total number of liver cells present in the autoradiograph and the number of labeled cells exclusive of the inflammatory cells was determined, and the labeled nuclei per 10,000 liver cells calculated. An attempt was made to differentiate the labelling of liver parenchymal, Kupffer, ductual and connective tissue cells.

XIII. Immunological Technics

A. Preparation of Rabbit anti-cat IgG antiserum: Saturated ammonia sulfate (25 ml) was slowly added to 50 ml normal cat serum. The mixture was stirred continuously in the cold overnight. The precipitated globulins were washed twice with 1/3 saturated ammonia sulfate. The precipitated globulins were free of hemoblogins. The precipitate was resuspended in 3 ml distilled water and dialyzed against normal saline in the cold for 24 hours (5 changes). The mixture was then dialyzed against 0.0175M phosphate buffer, pH 7.0 for 24 hours (3 changes). The globulins were placed on a DEAE cellulose column and chromatographically separated using the 0.0175M phosphate buffer. The fractions were collected on a LKB Ultrarac fraction collector 7000 at a rate of 80 drops per tube. The protein content of each fraction was determined using the Lowry-phenol method (Lowry et al., 1951) with BSA as a standard. Fractions 4--7, the first elution peak (see Figure 2), contained the isolated feline IgG. By pooling these four fractions, 20 ml solution containing 28 mg/ml IgG was obtained. The IgG was concentrated to 100 mg/ml. Antiserum to feline IgG was produced in a rabbit. The injection regime consisted of one injection subcutaneously of 0.5 ml isolated IgG mixed with 0.5 ml of complete Freunds adjuvant (Control No. 596533, Difco Laboratories, Detroit, Mich.) every three days for 2 weeks (5 injections) and 1 a week for 2 weeks (2 times). A trial bleeding showed a good antibody titer at four weeks so the rabbit was exanguinated at 4 1/2 weeks. Immunoelectrophoresis (Materials and Methods, XIII--D) of the rabbit antifeline IgG serum revealed only one band (see Figure





PREPARATION OF RABBIT ANTI-CAT IgG ANTISERUM

A. Graph A shows the ugm protein/ml for each fraction (80 drops) eluted from DEAE cellulose chromatographic column. Fraction 4--7 contain Cat IgG.

B Immunoelectrophoresis of normal cat serum in well 1 and isolated cat IgG in well 2. Rabbit anti-cat IgG was placed in the center trough.

FIGURE 2

THE ISOLATION AND PURIFICATION OF FELINE IgG

2) in the gamma migrating region.

B. Preparation of Rabbit anti-feline thymus: Thymuses from three neonate kittens were pooled and minced in saline. The lymphocytes (thymocytes) were washed 3 times with saline and treated with 0.83% ammonium chloride-Tris to remove residual erythrocytes. A portion of the thymic lymphocyte suspension was mixed with an equal volume of Freunds adjuvant and immediately injected subcutaneously into a rabbit. The remainder was frozen until needed and administered with the same regime used in preparing feline IgG (see Materials and Methods, XII-A). The rabbit anti-feline thymus serum was extensively absorbed with washed cat erythrocytes, normal cat serum and isolated feline immunoglobulins, according to the guidelines of Owens and Fanger (1974). No immunoelectrophoretic band was present between lymphocyte extracts and this antiserum, indicating the antibody produced was directed against membrane components and not soluble products.

C. Preparation of Rabbit Anti-Adult Platynosomum concinnum Serum: Approximately 1,000 adult P. concinnum were washed free of bile and homogenized in normal saline. Antiserum to this crude homogenate was produced in rabbits as described above (Materials and Methods XII-A). The same protocol was used to produce guinea pig anti-P. concinnum antiserum. The antiserum was directed mainly against the somatic antigens of the adult fluke.

D. Immunoelectrophoresis and Double Diffusion Technics: A measured quantity of 5 ml of 1.5% agarose suspended in barbital buffer of 0.05 ionic strength, pH 8.6 was applied to precleaned 2" x 3" glass slides. After the agarose had gelled, wells and troughs were cut,

5 lambda serum introduced into the wells, and electrophoresis of the slide was accomplished at 60 mV/slide for 90 minutes using cold barbital buffer in a Universal Electrophoresis Cell (Buchler Instruments, Fort Lew, N.J.). The troughs were filled with appropriate antiserum or antigen and incubated in a moist chamber at 10°C until bands formed. The slides were washed overnight in water to remove uncomplexed proteins, dried with filter paper, and stained with 2% aniline blue black (acid black 1, Matheson Coleman and Bell, Norewood, Ohio) in 7.5% acetic acid.

A modification of the standard Ouchterlony double diffusion gel technic was used (Ouchterlony, 1948). 2" x 3" precleaned slides were coated with 5 ml of 1.5% agarose suspended in saline citrate buffer. After filling the wells, the slides were incubated as described above.

E. Rosette Formation: The following procedures were used to study the number of B and T lymphocytes in cats.

1. Lymphocyte Isolation: 3 ml sample of heparinized blood was collected via venous puncture from each cat immediately prior to use. The buffy coat layer was mixed with 2 ml normal saline, carefully layered on 3 ml of Lymphoprep (Nyegaad & Co., AS. Oslo, Norway), and centrifuged at 1400 rpm for 30 minutes at 4°C. The lymphocytes were carefully washed twice in Hanks solution and resuspended at 2 x 10^6 lymphocytes per ml. Analysis of viability was carried out with the trypan blue exclusion test. All preparations showed greater than 95% viability. At least 90% of each preparation was lymphocytes; the remainder of the cells were mainly eosinophils with very few monocytes.

2. Test for EAC Rosettes (B cells): The procedure of Aiuti et al. (1973) for EAC rosette test was followed closely. A 5% suspension of sheep red blood cells (SRBC) was washed twice in Hanks solution and incubated at 37°C with the IgM fraction of rabbit anti-sheep RBC diluted 1:5000 for 1 hour. The cells were washed twice with Hanks and resuspended to a 5% solution. An equal volume of 1:20 diluted fresh cat plasma was added and incubated at 37°C for 1 hour. The cells were then washed twice and a final 0.5% solution was prepared in Hanks. Then 0.25 ml of the 0.5% SRBC suspension prepared above was mixed with 0.25 ml of a 2 x 10^6 lymphocytes per ml suspension in Hanks and incubated at 37°C for 15 minutes. The cells were centrifuged at 200x g for 2 minutes. Most of the supernatant was removed and the cells resuspended by vigorous mixing. An aliquot of cells placed under a suspended coverslip and at least 300 lymphocytes were counted. Lymphocytes with 3 or more SRBC bound were considered to be positive for EAC rosettes.

3. Test for E Rosettes (T cells): The procedure of Taylor et al. (1975) was followed for all E rosettes studies. Defibrinated blood was collected from guinea pigs and used immediately or stored in Alsever's solution. The guinea pig red blood cells (GPRBC) were washed twice in saline and a 0.5% suspension was prepared in Hanks. A 0.25 ml suspension of each RBC solution was mixed with 0.25 ml of 2 x 10^6 lymphocytes/ml and incubated 15 minutes at 37°C. The cell suspension was then centrifuged at 300x g for 2 minutes. The tubes were stored overnight at 4°C. Most of the supernatant was removed, the cells were resuspended by gentle shaking. At least 300 lymphocytes were counted

and those with 3 or more bound guinea pig erythrocytes were considered to be positive for E rosettes.

F. Fluorescent Antibody Studies:

1. Preparation of Fluorescent Labeled Antisera: A portion of rabbit anti-feline IgG and thymus (anti-T) antisera was labeled with fluorescein isothiocyanate according to the procedure of Croxatto et al. (1968). Fluorescein isothiocyanate (25 μ g/mg serum protein) was added to antiserum whose pH had been adjusted to pH 9.5 with 0.1N sodium hydroxide. After incubation at 10°C overnight, the free isothiocyanate was separated from that bound to the globulins by chromatography through a SG-25 column (36 x 4 cm) using a 0.01M phosphate buffer, pH 7.4.

2. Immunofluorescent Lymphocyte Studies: Isolated lymphocytes (2 x 10^6/ml) (Materials and Methods, XIII--E--1) were incubated with an equal volume of fluorscein conjugated antiserum (rabbit anti-feline IgG or rabbit anti-feline thymus) for 20 minutes at 37°C. The cells were washed twice with 0.05M phosphate buffer pH 8.1, and resuspended in 50% glycerol in barbital buffer. Cells were examined with a Leitz Dialux microscope equipped with a 200W ultra-high pressure mercury lamp. A K510 suppression filter was used. Two hundred lymphocytes were counted per sample. The majority of the fluorescent cells showed patchy staining; a few cells had diffuse peripheral fluorescence.

G. The Effect of Mitogens on Blast Transformation: The effect of various mitogens on feline lymphocytes was studied. Buffy coat cells (0.1 ml containing 6.0 x 10^5 lymphocytes) or 0.1 ml of isolated lymphocytes (2 x 10^6 lymphocytes/ml) were added to 25 x 75 mm sterile

Falcon plastic tubes which contained 0.2 ml tissue culture (TC) medium, 0.1 ml autologous plasma, and either a) 0.1 ml phytohemagglutinin (1:3 dilution, Difco-Bacto-Phytohemagglutinin M, Difco Laboratories, Detroit, Mich.) or b) Pokeweed Mitogen (1:10 dilution, Barker and Farnes, Grand Island Biological Co., Berkeley, Cal.), or c) Concanavalin A (25 μgm/ml, Calbiochem, San Diego, Cal.), or d) various concentrations of Platynosomum concinnum antigen (10% extract of adult flukes homogenized in a solution of fluke metabolic products), or e) sterile saline.

Tissue culture medium consisted of 10% Eagle's basal medium (BME)-Hanks 10% fetal calf serum (Decomplemented), 1.0% glutamine, streptomycin 100 U/ml with the pH adjusted to 7.4 using sterile 7.5% sodium bicarbonate.

The tissue culture mixtures and appropriate controls were incubated for 3-5 days at 37°C without disturbing the cells. Tritiated thymidine containing 0.5 μ Ci (specific activity 1.0 Ci/nM, International Chemical and Nuclear Corp., Irvine, Cal.) in 0.05 ml sterile saline was added to each tube 24 hours prior to harvesting of the cells.

At the end of the incubation period, the cells in the tubes were mixed thoroughly and 0.1 ml sterile cold thymidine (1000x) was added to each tube. The tubes were then shaken thoroughly to insure a uniform suspension and 0.1 ml was removed from each tube with a micropipet and placed on a 2.3 cm diameter Whatman 3MM filter paper circle (W & R Balston, Ltd., England). The discs containing the samples were air-dried, placed in a 500 ml flask and washed with three

changes of 250 ml 5% cold trichloroacetic acid solution at 15 minute intervals. Following one wash in each of 200 ml of 95% ethyl alcohol, ethyl alcohol-ether (50% mixture), and ether, the paper discs were air-dried and placed in liquid scintillation counting vials. To each vial was added 5 ml of Omnifluor in toluene (98% PPO and 2% Bis-MSB, New England Nuclear, Boston, Mass.).

The samples (prepared in duplicate) were counted for 5 minutes at 4°C in a Packard Tri-Carb Scintillation Spectrometer. After correction for background, the ratio of counts between experimental and control values were determined.

H. Procedure for Radiommunoabsorbance to Detect Blocking Antibodies on the Surface of the Liver Fluke: Whole adult Platynosomum concinnum which had been frozen for antigen were washed three times with saline. Half of the flukes were incubated in rabbit-antiadult fluke serum and half were incubated in normal rabbit serum for 3 hours at 37°C. The flukes were washed in saline five times and incubated in ^{125}I labelled goat anti-rabbit IgG for 3 hours. The flukes were washed 5 times in saline and the activity was monitored in a Gamma 300 system counter (Beckman Laboratory Instrumentation, Fullerton, Cal.).

I. Method for the Evaluation of the Role of Cellular Immunity in Platynosomiasis: Seven cats were used in this study. The donor cat was No. 27, Group C, female. All six of these 1 year old recipient cats had the same paternity; three were from a litter of cat No. 27 who was the mother of cats B, E and F, while the other three were from a litter of an unknown queen.

Cat No. 27 was sacrificed 187 days post-infection. Mesenteric lymph nodes and spleens were removed at the time of necropsy. They were minced in Hanks solution and washed free of particulate matter. An innoculum of 1.32×10^6 B lymphocytes, 5.8×10^6 T lymphocytes and 5.0×10^6 null-cells from the mother cat were injected intraperitoneally into each of two of her kittens (cats E and F) within 3 hours of collection. The cats received 1 ml bicillin at the time of lymphocyte transfer.

Cats C and D received 20 ml serum from the donor cat I.P. and 1 ml bicillin. The other two cats A and B, served as controls and received saline I.P. only.

Three days after lymphocyte transfer, all 6 cats were infected with platynosomum. One hundred infective stages were isolated from toad livers and passed via stomach tube into each cat. Approximately 4 hours were required to complete the transfer of the infectious material.

Five days after infection these cats were injected subcutaneously with Toxocara cati antigen in Freunds complete adjuvant in order to determine if the transferred lymphocytes had remained viable in the recipient animals. The donor cat had been immunized with this antigen.

The cats were bled for hematological evaluation according to the time schedule shown in Figure 3.

All 6 cats were sacrificed and necropsied 8 weeks post-infection. Standard necropsy procedures were followed.

Week No.	Days after Infection						
-1					-3 #27 PTS - #E, F Received Lymphocytes		-1 C, D Received Serum
0	+1 All cats infected with 100 infective stages	2	3	4	5 All 6 cats bled - injected Ag marker	6	7
1	8 Bled 6 cats	9	10	11	12	13 Bled 6 cats	14
2	15	16	17	18	19	20 Bled 3 cats	21
3	22	23	24	25	26	27 Bled 3 cats	28
4	29	30	31	32	33	34 Bled 3 cats	35
5	36	37	38	39	40	41 Bled 3 cats	42
6	43	44	45	46	47	48 Bled 3 cats	49
7	50	51	52	53	54	55 Bled 3 cats	56
8	57	58	59	60	61 PTS B, C, E	62	63 PTS A, D, F

FIGURE 3

SCHEDULE FOR IN VIVO STUDY ASSESSING THE
ROLE OF CELL MEDIATED IMMUNITY IN PLATYNOSOMIASIS

RESULTS

1. Clinical Signs and Symptoms

During the nine months of experimental infection with P. concinnum, each cat was examined daily for signs of illness. In general, the health of the animals remained excellent. With the exceptions presented below, all cats had shiny coats, good appetites and playful dispositions.

No signs of ill health were noted in Group A (control), Group B (low dose) or Group D (long-term) animals. However, five of eight Group C (high dose) cats presented clinical signs and symptoms attributable to P. concinnum infection. Figure 4 lists the observed clinical signs and symptoms with time. Seven to sixteen weeks post-infection these 5 Group C (high dose) cats displayed lethargy, inappettence, and abdominal tenderness. Fever was not present. The animals did not display clinical signs of jaundice despite elevation of serum icterus index to 10 or more units. Minor weight changes occurred during this time. (See Appendix A for the weight chart of each cat.) All five cats then recovered and remained in apparent good clinical health.

Several authors (Puruis, 1933; Catcott, 1964) have raised the question whether platynosomiasis caused weightloss and emaciation. The weight changes for both male and female Group A, B and C animals are displayed in Figure 5. The weight of each cat as well as average weights for each group with standard deviation are listed in Appendix A. Although all animals were considered to be adult at the beginning

Cat	Time (weeks) post-infection	Sign and/or Symptom Noted
Cat #26 (female)	0	Infected with 1952 infective stages-- in estrus
	11	Inappetence, weight loss
	12-15	Lethargy, with continued weight loss
	16	Euthanised: 42.5% of experimental infective dose recovered
Cat #27 (female)	0	Infected with 1978 infective stages
	8	Normal body temperature, lethargy pendulus abdomen, slight liver enlargement, walks as though abdominal pain
	9	Improvement
	12-41	Return to normal clinical health
	42	Euthanised: 10% of experimental infective dose recovered
Cat #29 (female)	0	Infected with 1978 infective stages
	7	Lethargic
	8	Slightly distended abdomen, walks slowly, very weak
	11	Great physical improvement
	14	Return to normal health
Cat #23 (male)	0	Infected with 1978 infective stages
	11	Inappetence
	15	Lethargy, intermittent vomiting
	16	Improvement
	17	Relapse with lethargy and weight loss
	19	Abdominal tenderness
	20-25	Slight lethargy
	26-36	Improvement
	37	Euthanised: 60.5% of experimental infective dose recovered
Cat #22 (male)	0	Infected with 1978 infective stages
	8	Inappetence
	9	Chromic urine
	11	Nasty disposition, not feeling well, slight weight loss
	16	Return to normal health
	37	Euthanised: 23.1% of experimental infective dose recovered

FIGURE 4

SIGNS AND SYMPTOMS OF 5 GROUP C CATS WITH EXPERIMENTALLY INDUCED P. CONCINNUM INFECTION

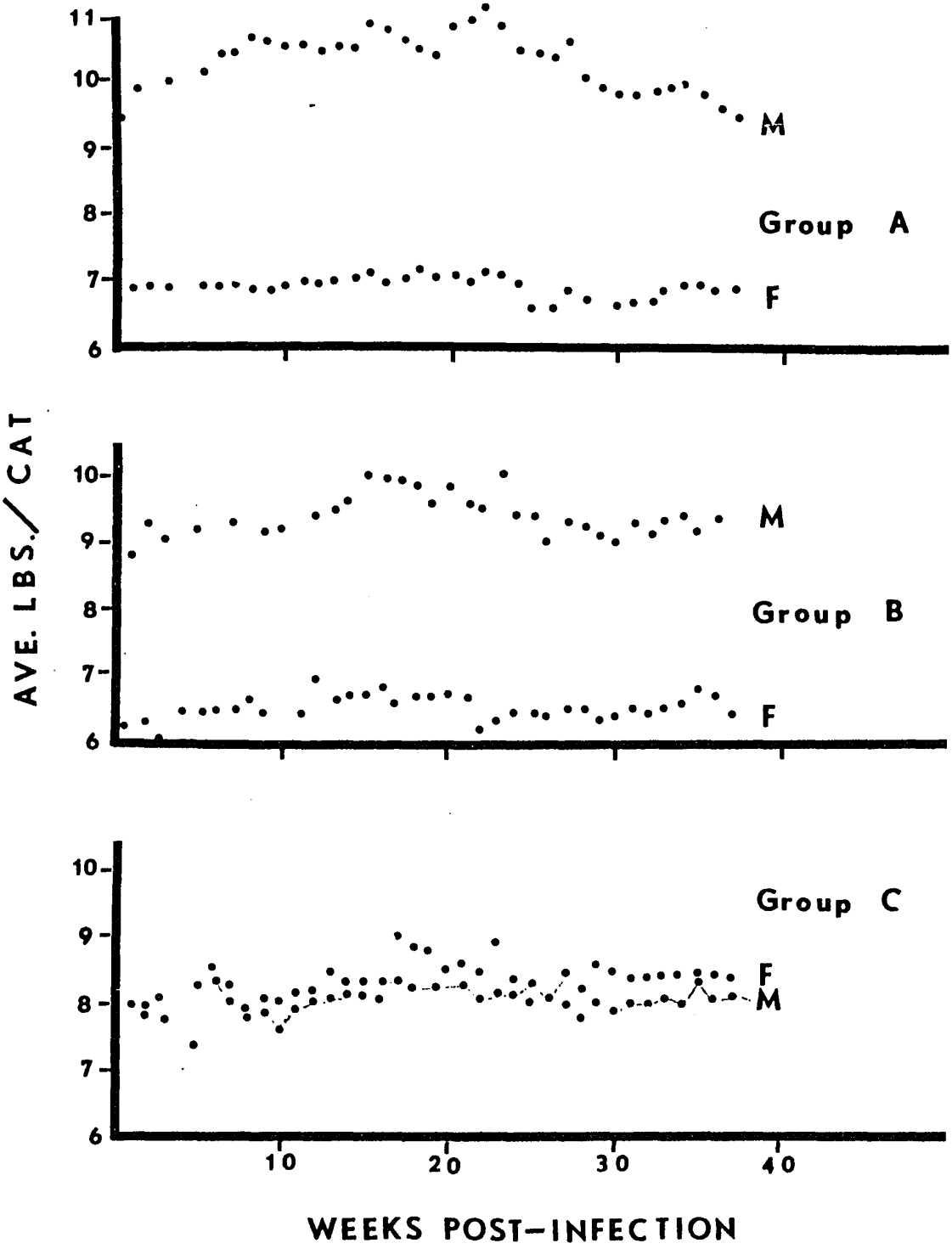


FIGURE 5

THE AVERAGE WEIGHT OF MALE (M) AND FEMALE (F) CATS FOLLOWING P. CONCINNUM INFECTION

of the experiment, some cats had not yet attained full adult size; considerable variation was therefore noted in the amount of weight gained by individual cats. There was no weight loss in any of the experimentally infected animals during this 9 month study. All cats gained weight except for a female control cat (#8) and a female low dose cat (#19).

Neither vomiting nor diarrhea were specifically associated with platynosomum infection. Vomiting was infrequent and occurred equally in Group A, B and C animals. Two animals (#13, #29) consistently passed soft stools, prior to experimental infection and throughout the course of disease. All other cats had feces of normal consistency.

2. Results of Hematologic, Biochemical and Serological Studies

All animals had normal hematologic profiles at the time of liver biopsy and subsequently prior to experimental infection (see Appendix B).

The first hematologic change noted was an increase in the number of eosinophils in the peripheral circulation. Figures 6 and 7 show the eosinophilia noted for Group A, B and C male and female cats as a function of time. Eosinophilia was apparent in Group B females and Group C males and females 3 weeks after infection. Eosinophilia was also noted in low dose males 14 weeks after infection. The eosinophilia in general remained high throughout the following 9 month period but tended to diminish with time during the infection. Data for each animal is listed in the appendix.

The solid, horizontal line in the graphs represent the upper

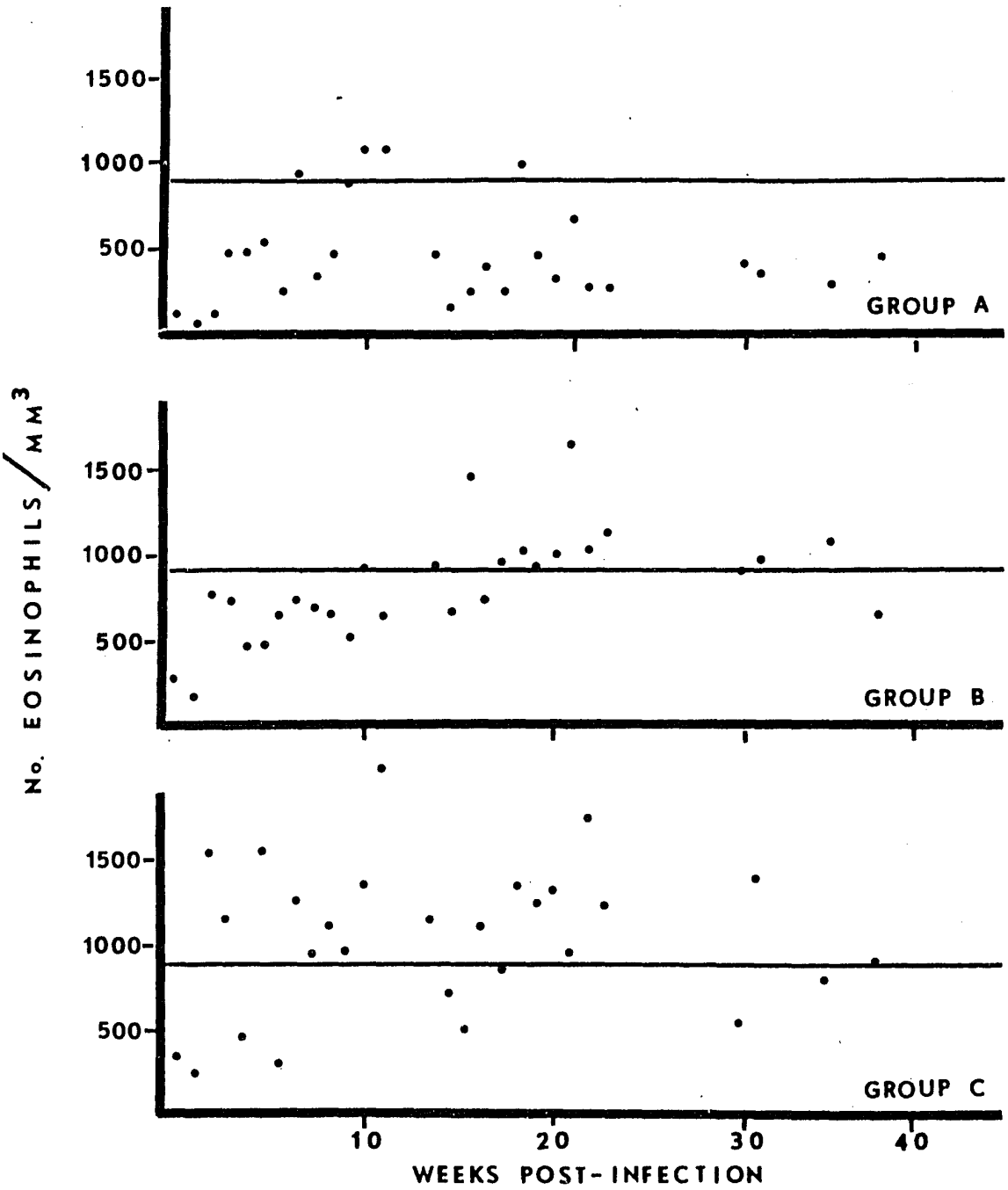


FIGURE 6

NUMBER OF EOSINOPHILS PER CUBIC MILLIMETER OF PERIPHERAL BLOOD IN MALE CATS FOLLOWING P. CONCINNUM INFECTION

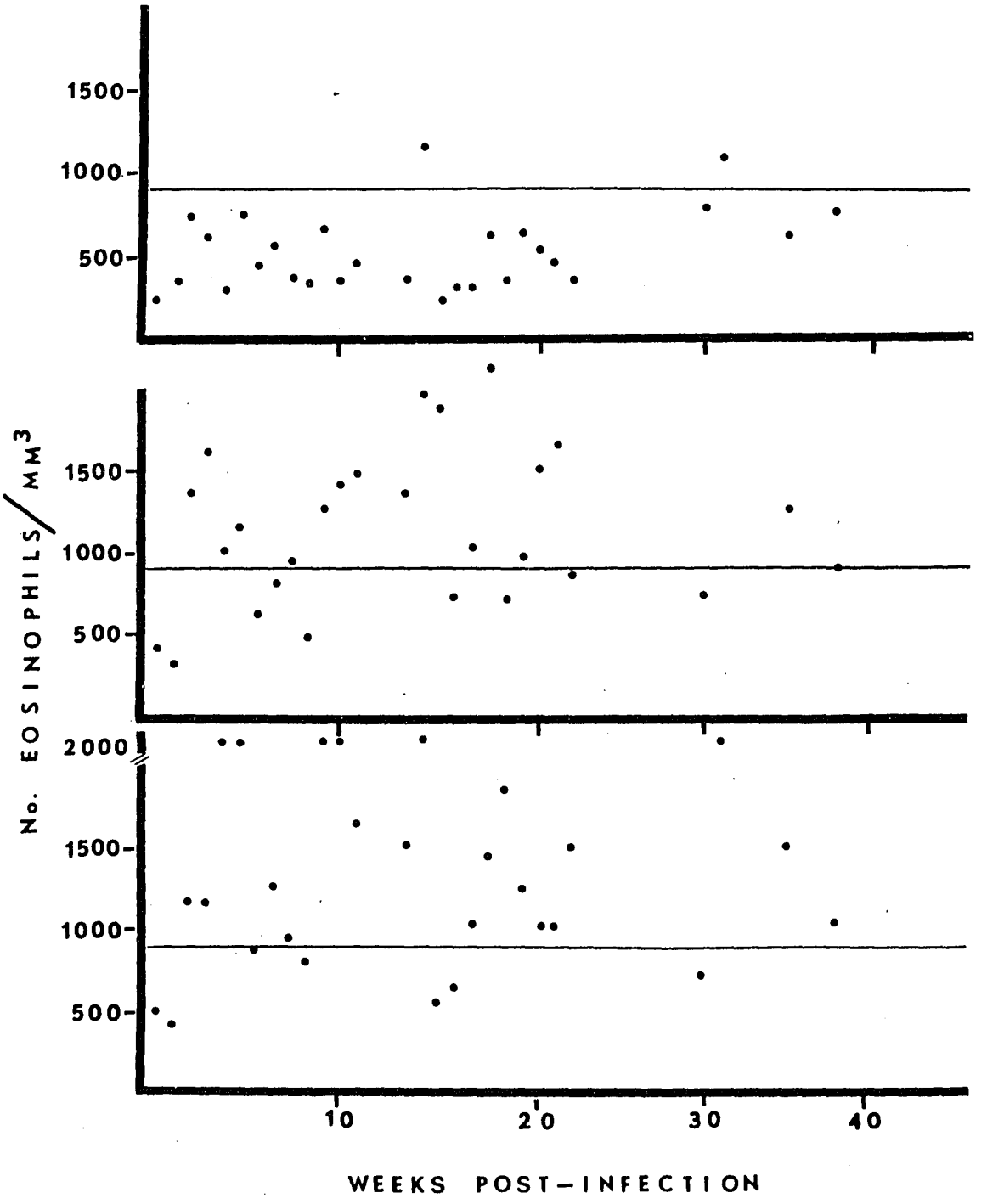


FIGURE 7

NUMBER OF EOSINOPHILS PER CUBIC MILLIMETER OF PERIPHERAL BLOOD IN FEMALE CATS FOLLOWING P. CONCINNUM INFECTION

limit of the range for normal animals.

The second hematologic change observed was the elevation of serum transaminase enzyme activity during the course of experimental infection. An increase in serum glutamic pyruvic acid transaminase enzyme activity occurred in Group C females 3 weeks, and in Group C males 7 weeks after infection. A very slight stimulation in SGPT was present in Group B animals. The average SGPT values for male and female animals are presented in Figures 8 and 9. Three months after infection this serum transaminase enzyme had returned to normal levels and remained constant thereafter.

An increase in serum glutamic oxaloacetic acid transaminase enzyme activity was also noted. A significant elevation was observed by 3 weeks after infection in Group C females and 10 weeks after infection in Group C males; a very small increase during the course of the disease was noted in Group B animals. This enzyme activity also returned to normal levels within 3 months. These trends can be seen in Figures 10 and 11 which also note the average enzyme levels for Group B and C males and females. The enzyme levels for each animal are listed in the appendix.

Alkaline phosphatase enzyme levels did not become elevated in parasitized animals during the period of study; however, an elevated icterus index was noted in four Group C cats (#22, 26, 27, 28) 8--12 weeks post-infection (Figure 12). The elevation in icterus index corresponded to the time period when clinical signs and/or symptoms were observed in these animals. Cats number 22, 23, 28 and 29 had abnormally high serum total bilirubin levels 7--16 weeks after

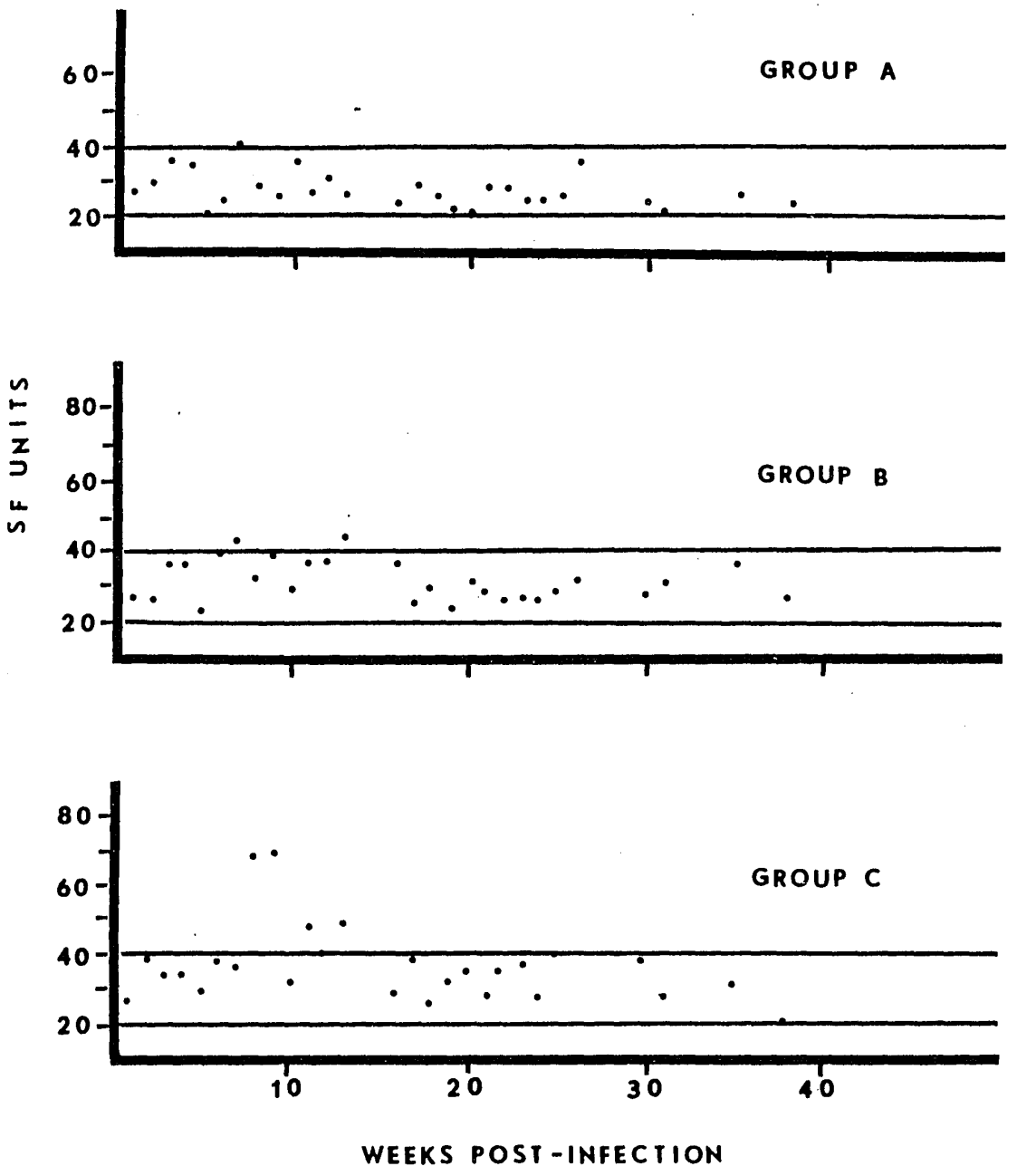


FIGURE 8

SERUM GLUTAMIC PYRUVIC ACID TRANSAMINASE (SGPT) ENZYME LEVELS IN MALE CATS DURING P. CONCINNUM INFECTION

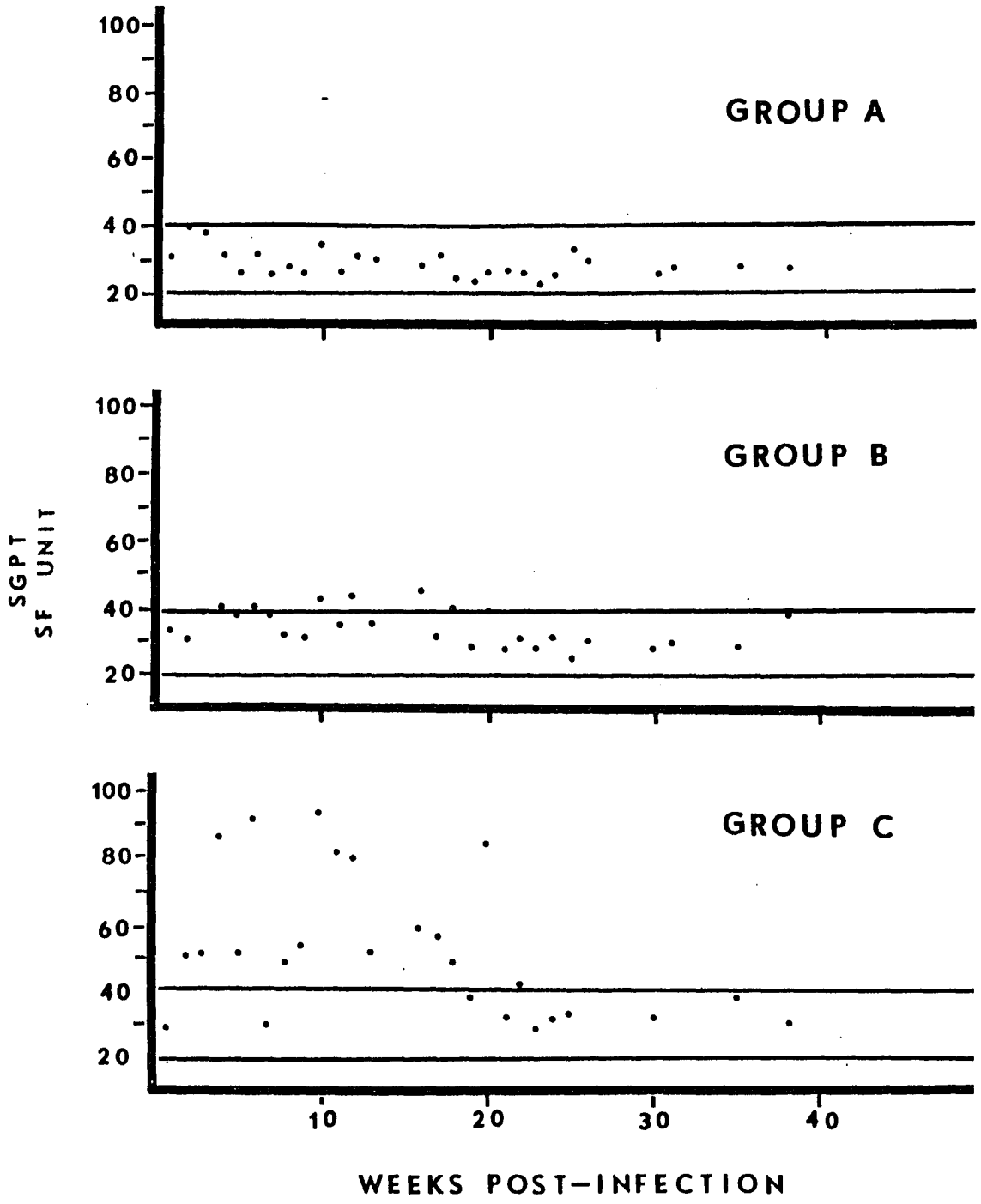


FIGURE 9

SERUM GLUTAMIC-PYRUVIC ACID TRANSAMINASE (SGPT) ENZYME LEVELS IN FEMALE CATS DURING P. CONCINNUM INFECTION

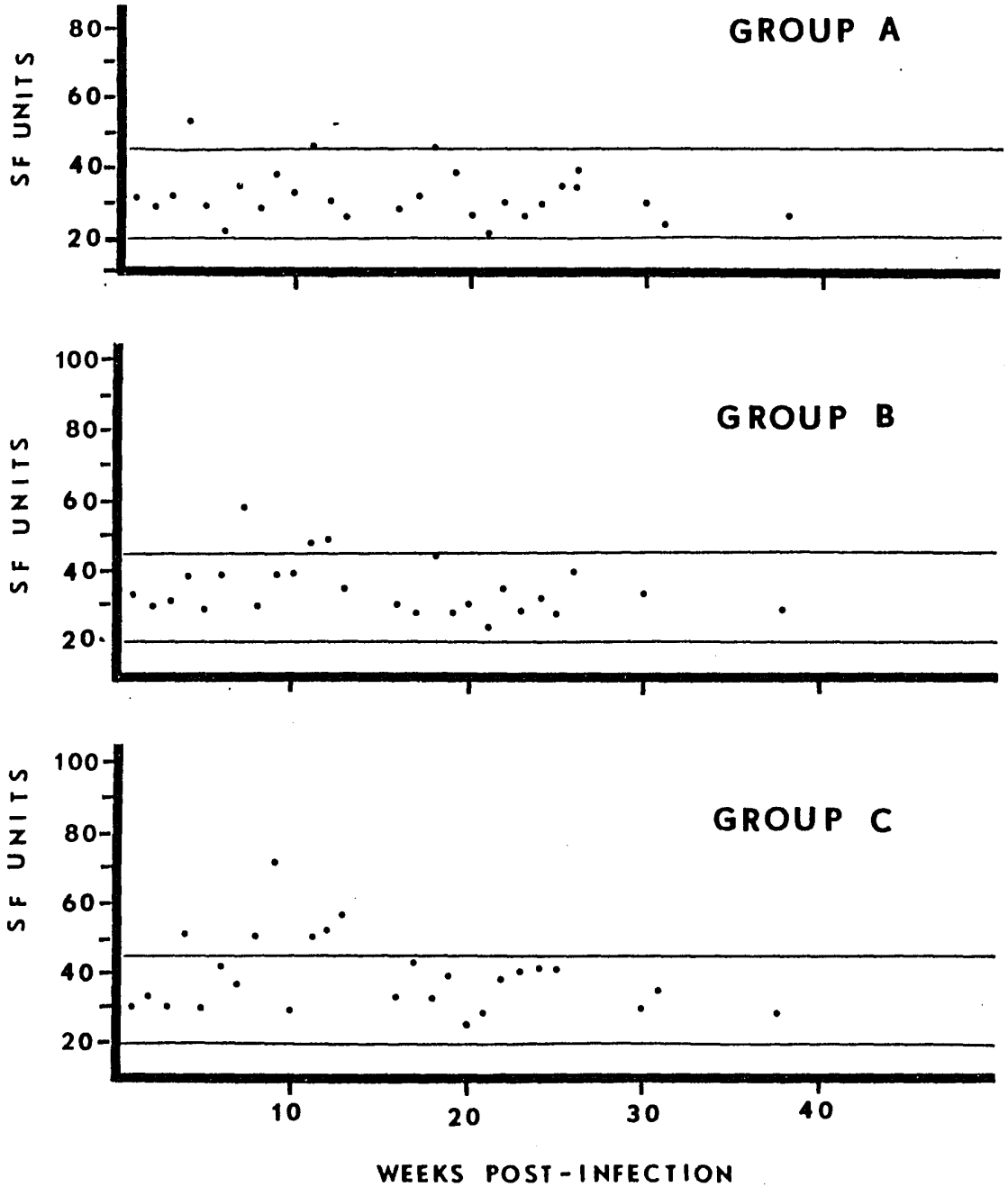


FIGURE 10

SERUM GLUTAMIC-OXALOACETIC ACID TRANSAMINASE (SGOT) ENZYME LEVELS IN MALE CATS DURING EXPERIMENTAL *P. CONCINNUM* INFECTION

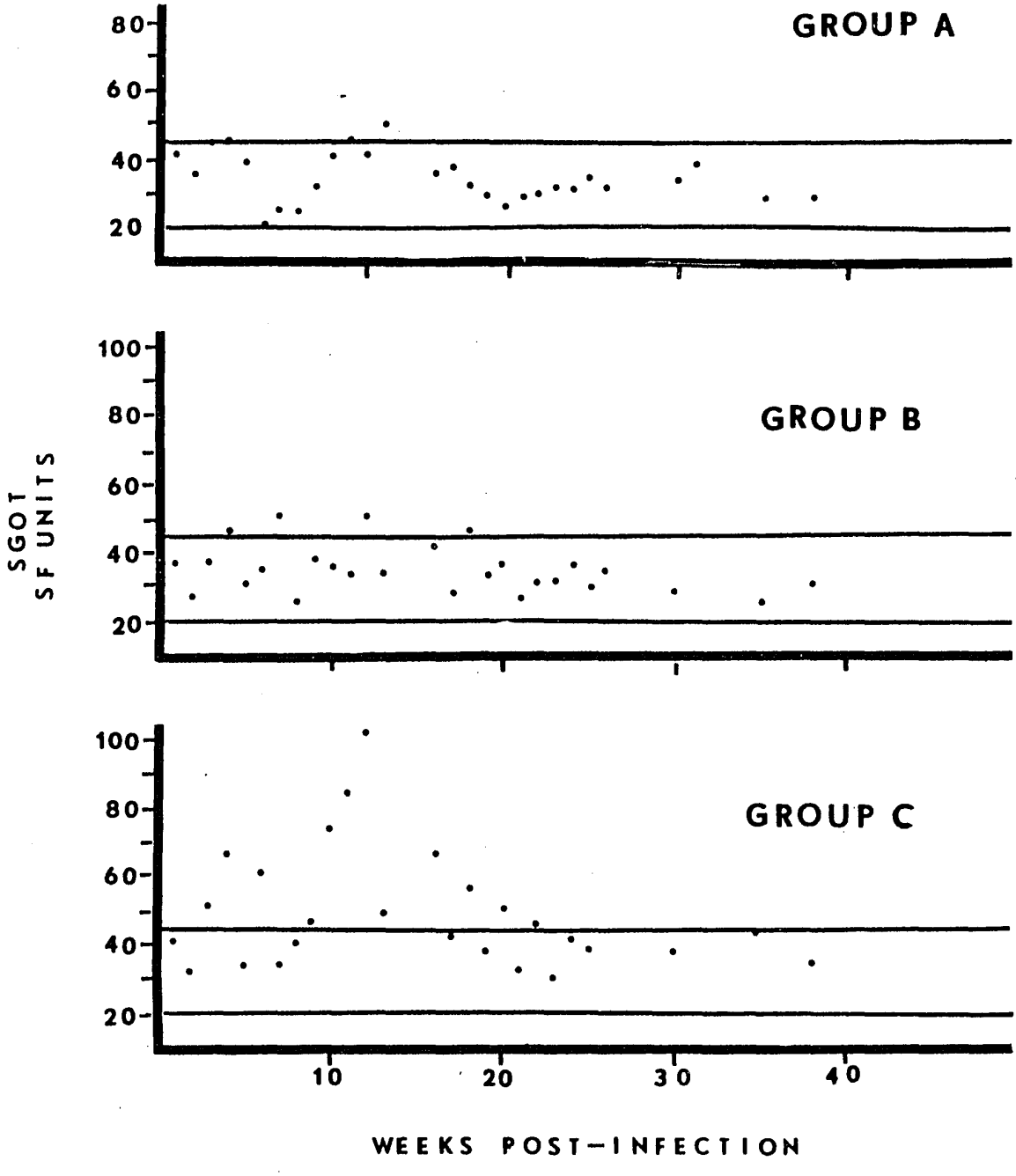


FIGURE 11

SERUM GLUTAMIC-OXALOACETIC ACID TRANSAMINASE (SGOT) ENZYME LEVELS IN FEMALE CATS DURING EXPERIMENTAL *P. CONCINNUM* INFECTION

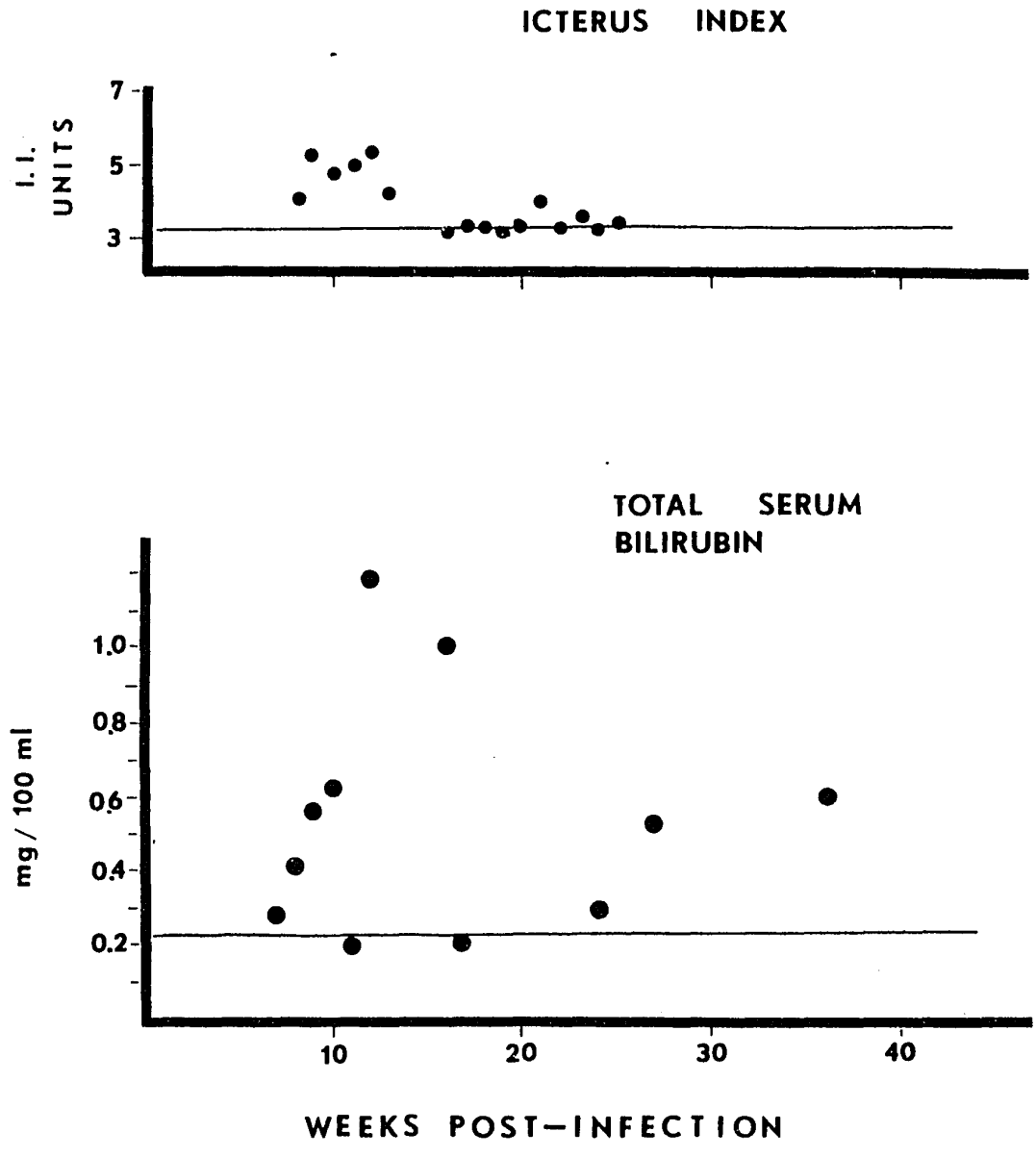


FIGURE 12

SERUM ICTERUS INDEX AND TOTAL BILIRUBIN VALUES
IN GROUP C (HIGH DOSE) CATS DURING
P. CONCINNUM INFECTION

experimental infection (Figure 12). All other cats had normal icterus index and total serum bilirubin values throughout the course of infection (Appendix B).

Seven hematologic tests were performed on blood samples to detect the possible presence of anemia. The results from packed cell volume, erythrocyte count, hemoglobin content, sedimentation rate, MCH, MCV and MCHC analyses are summarized in Figures 13 and 14. The mean erythrocyte diameter remained less than 5u throughout the period of study in control and parasitized cats. All values recorded for these hematologic parameters remained within the normal ranges for felines. Signs of anemia were not revealed by these technics.

The serum protein, albumin and globulin values are presented in Figure 15. Figure 16 displays the serum ions and Figure 17 shows the total leucocyte differential count and blood urea nitrogen findings. As can be seen, these values remained constant in infected animals during the period of study. All of the above values remained stable throughout the study. The hematologic data for each cat is tabulated in the Appendix.

Wide variation in hematologic and serum biochemical values occurred in cats receiving the same infective dose. For example, selected blood parameters are compared for two Group C females (#28 and 29). The hematological profile of cat #28 is shown in Figure 18. SGPT enzyme activity peaked at week 4 while SGOT enzyme activity peaked twice, at weeks 4 and 12. A slight eosinophilia was present which reached its maximum in week 31. The initial WBC for cat #28 was relatively high, but then tended toward leucopenia during the first

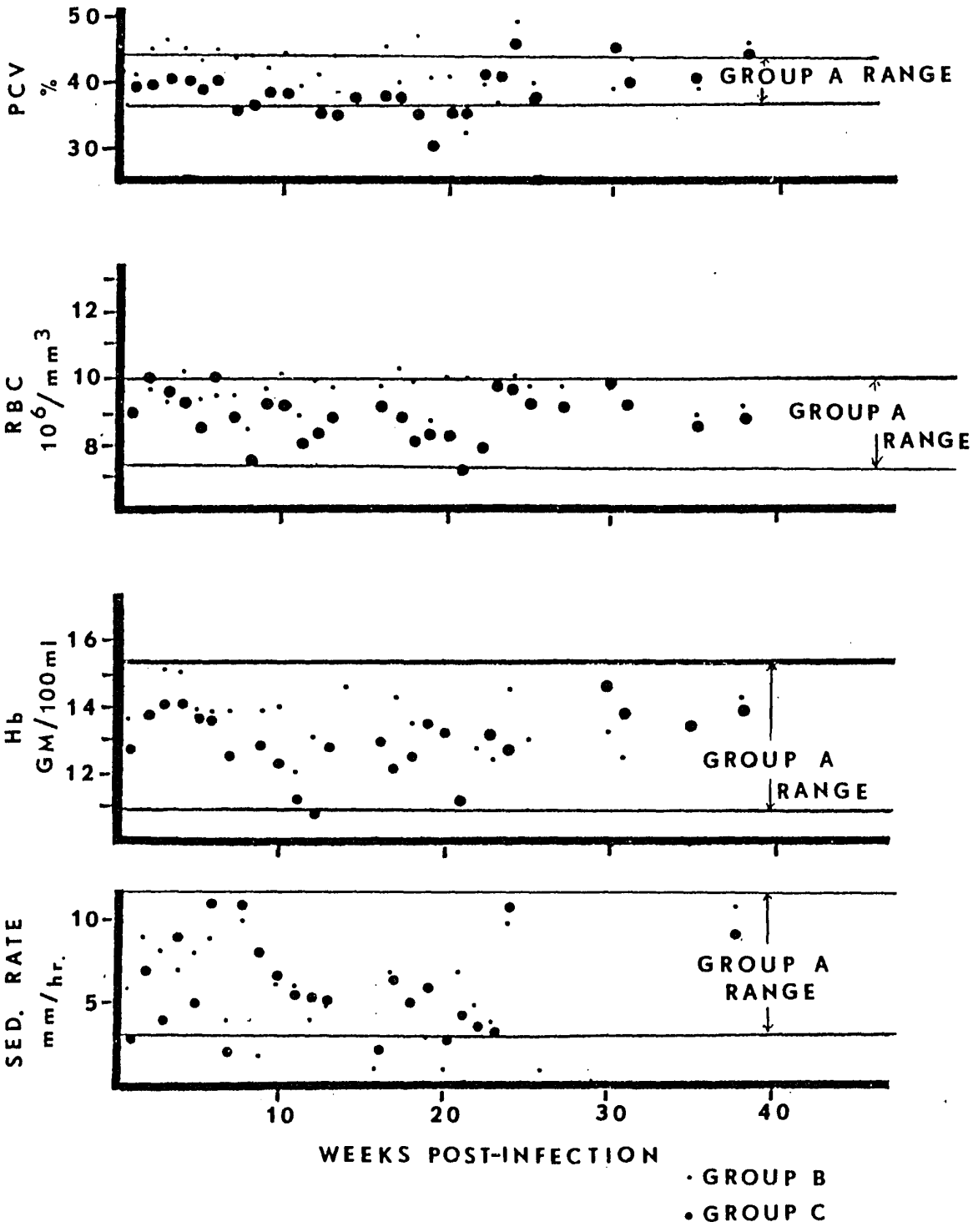


FIGURE 13

PCV, ERYTHROCYTE COUNT, HEMOGLOBIN CONTENT, AND SEDIMENTATION RATE FOR GROUP B & C CATS DURING P. CONCINNUM INFECTION

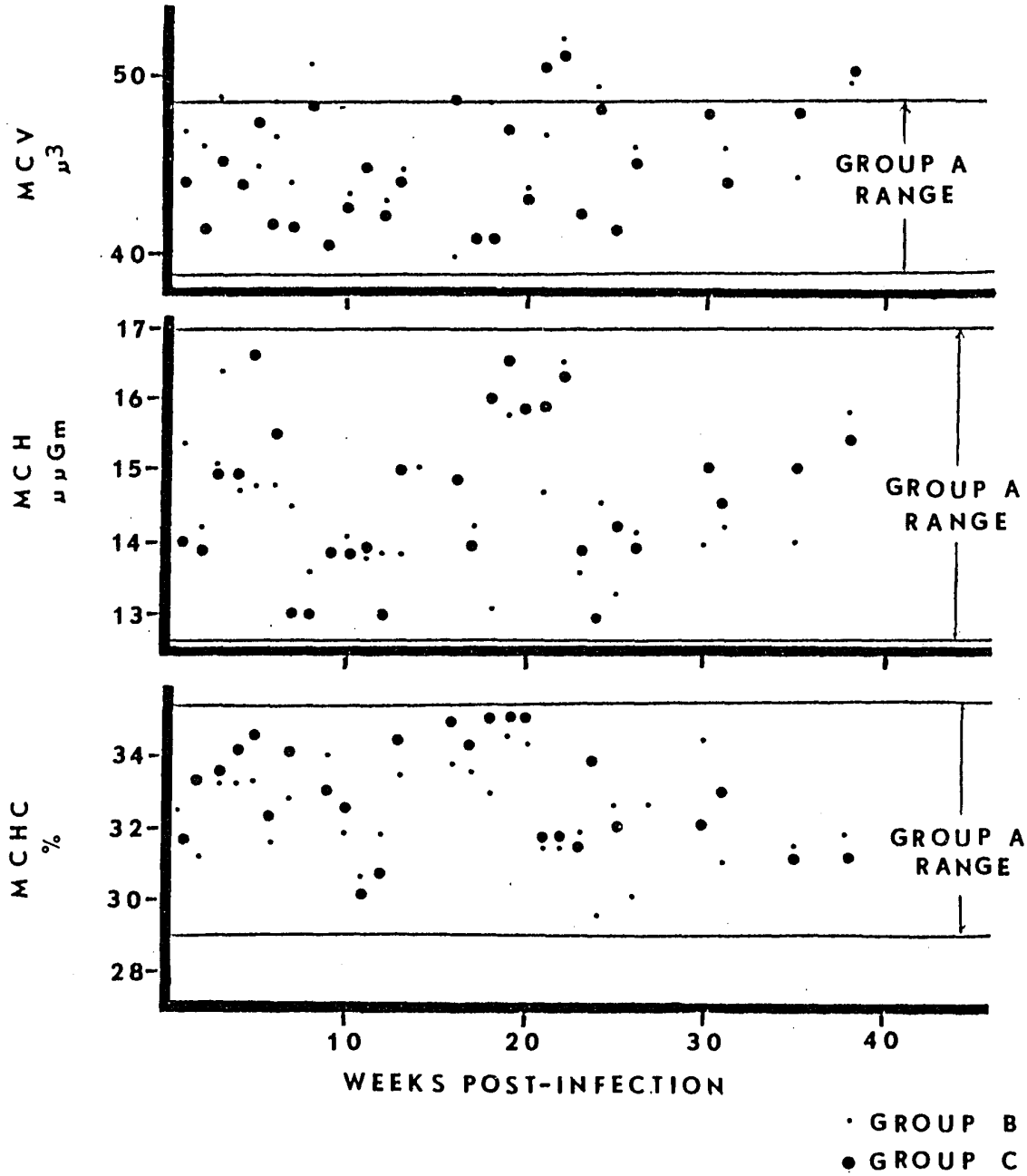


FIGURE 14

MCV, MCH, AND MCHC VALUES FOR GROUP B & C CATS
DURING P. CONCINNUM INFECTION

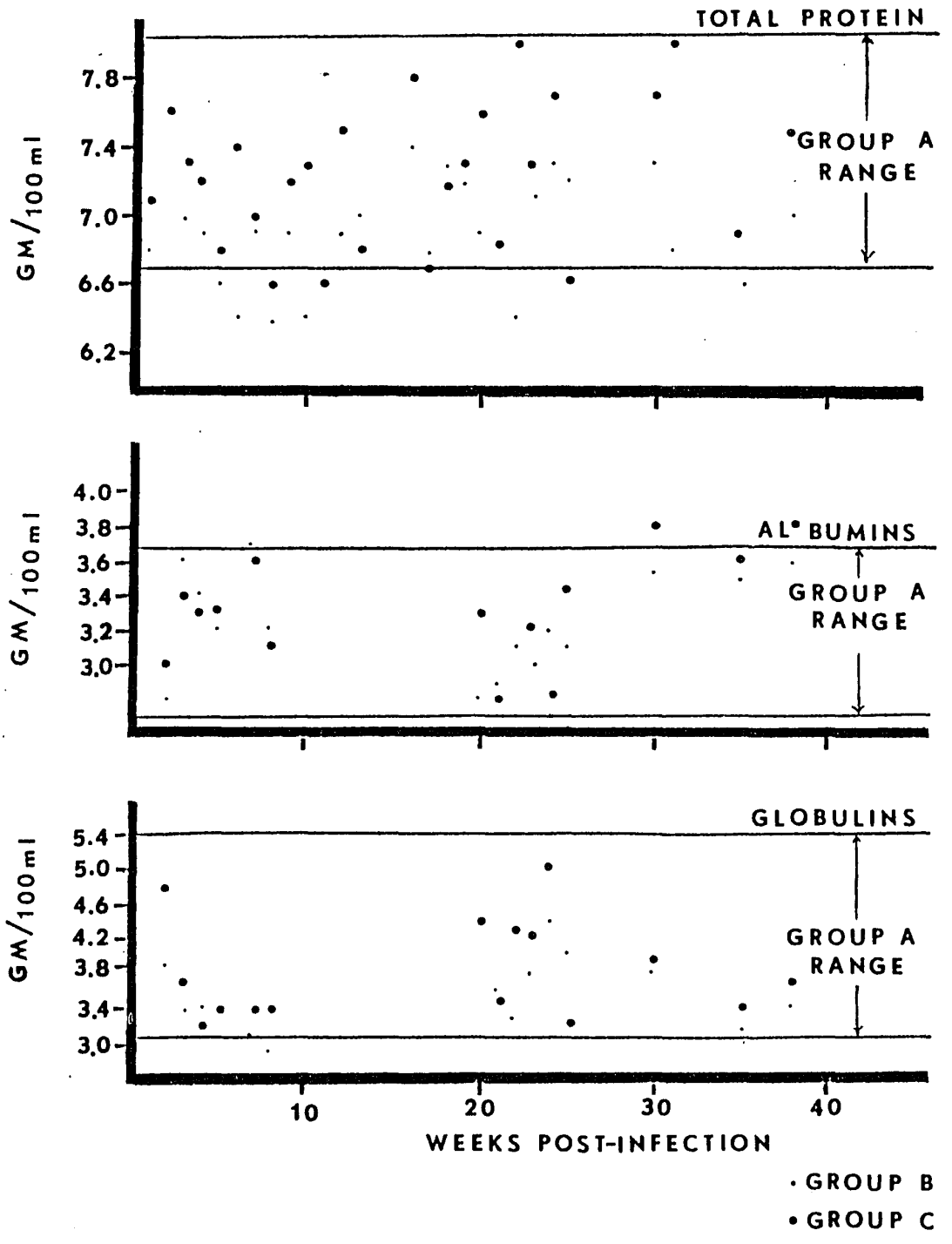


FIGURE 15

TOTAL SERUM PROTEIN, ALBUMIN, AND GLOBULIN VALUES
FOR EXPERIMENTALLY INFECTED CATS

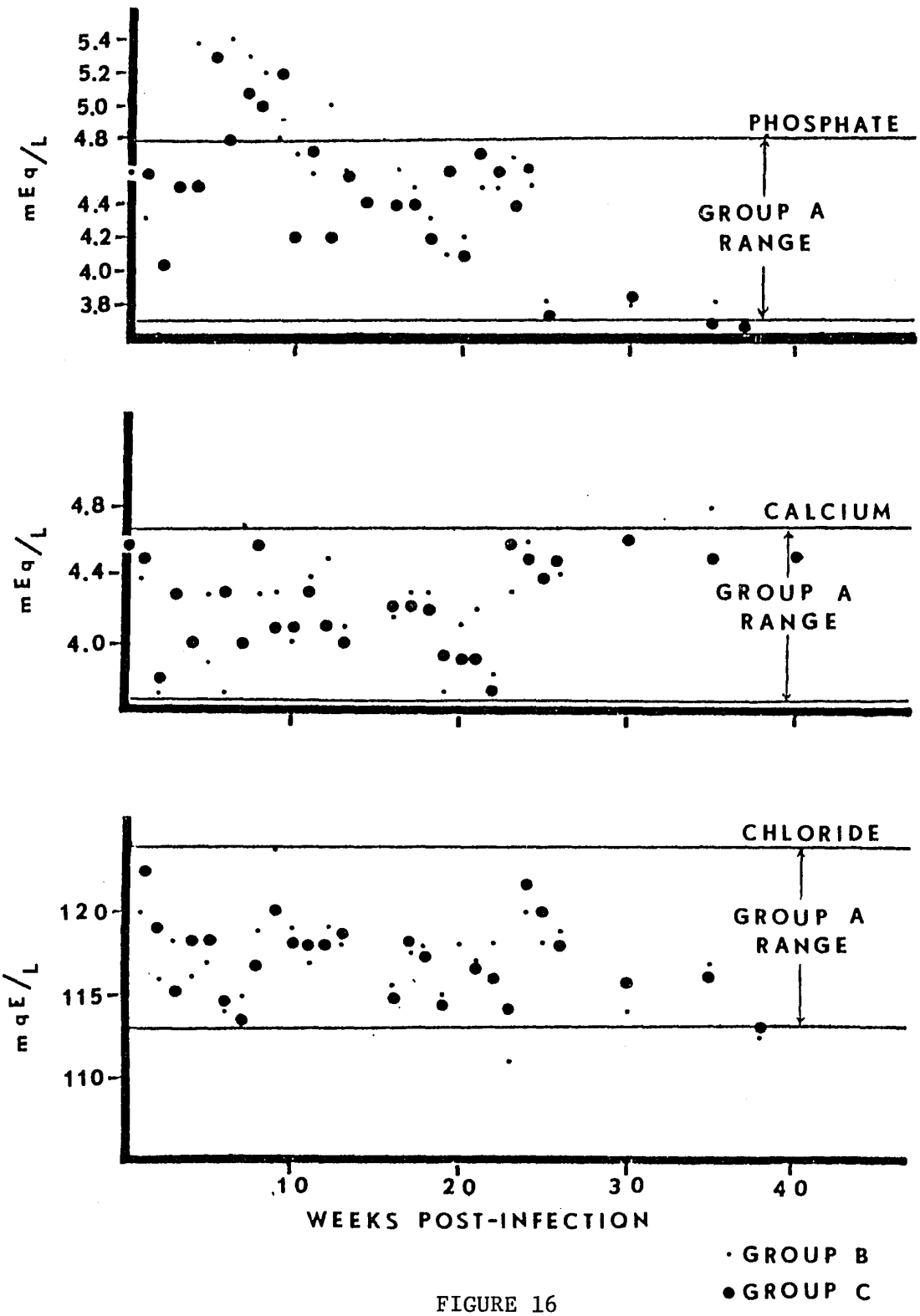


FIGURE 16

THE CONCENTRATION OF SERUM IONS
IN EXPERIMENTALLY PARASITIZED CATS

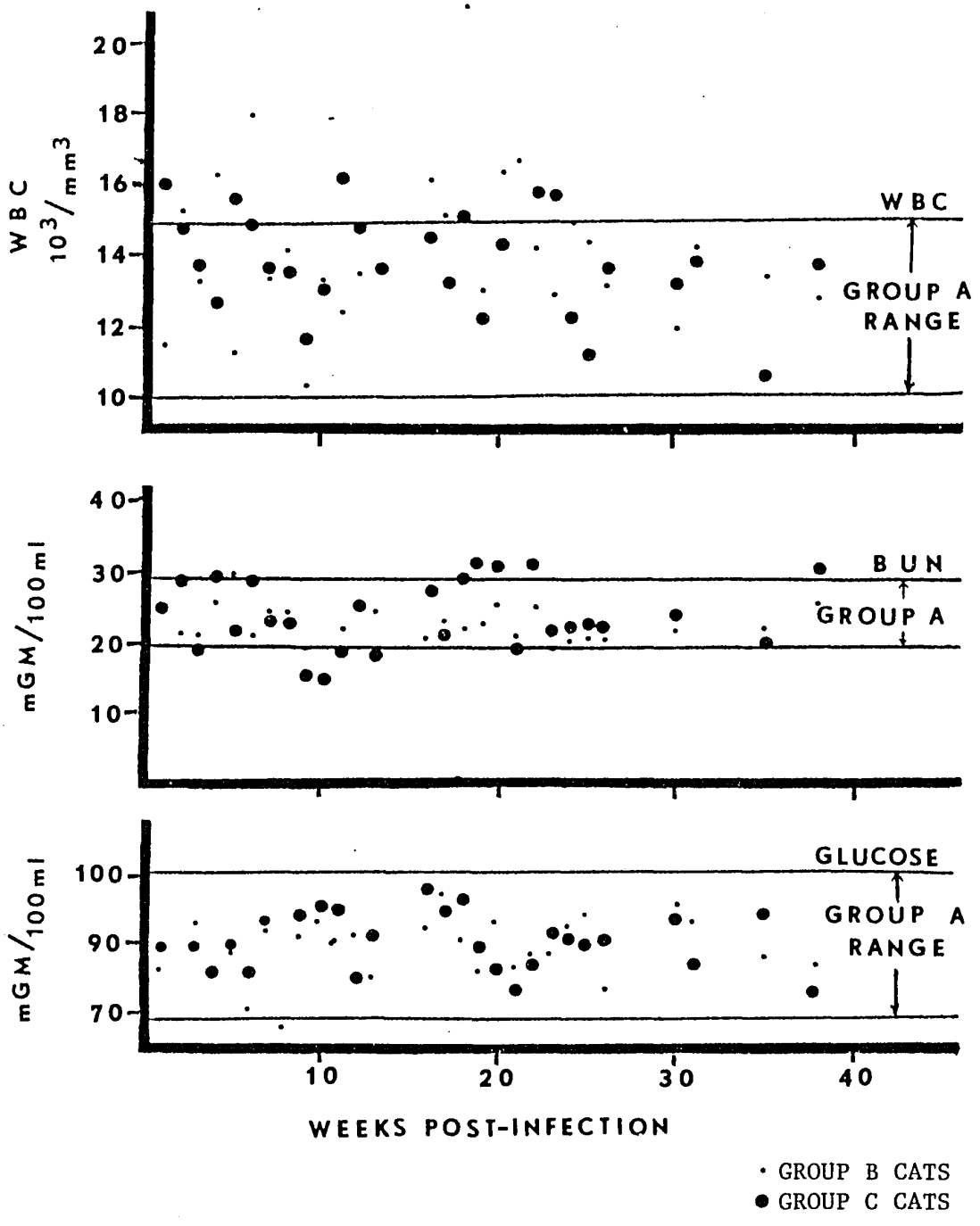


FIGURE 17

WHITE BLOOD CELL, GLUCOSE, AND BUN STUDIES
IN EXPERIMENTALLY INFECTED CATS

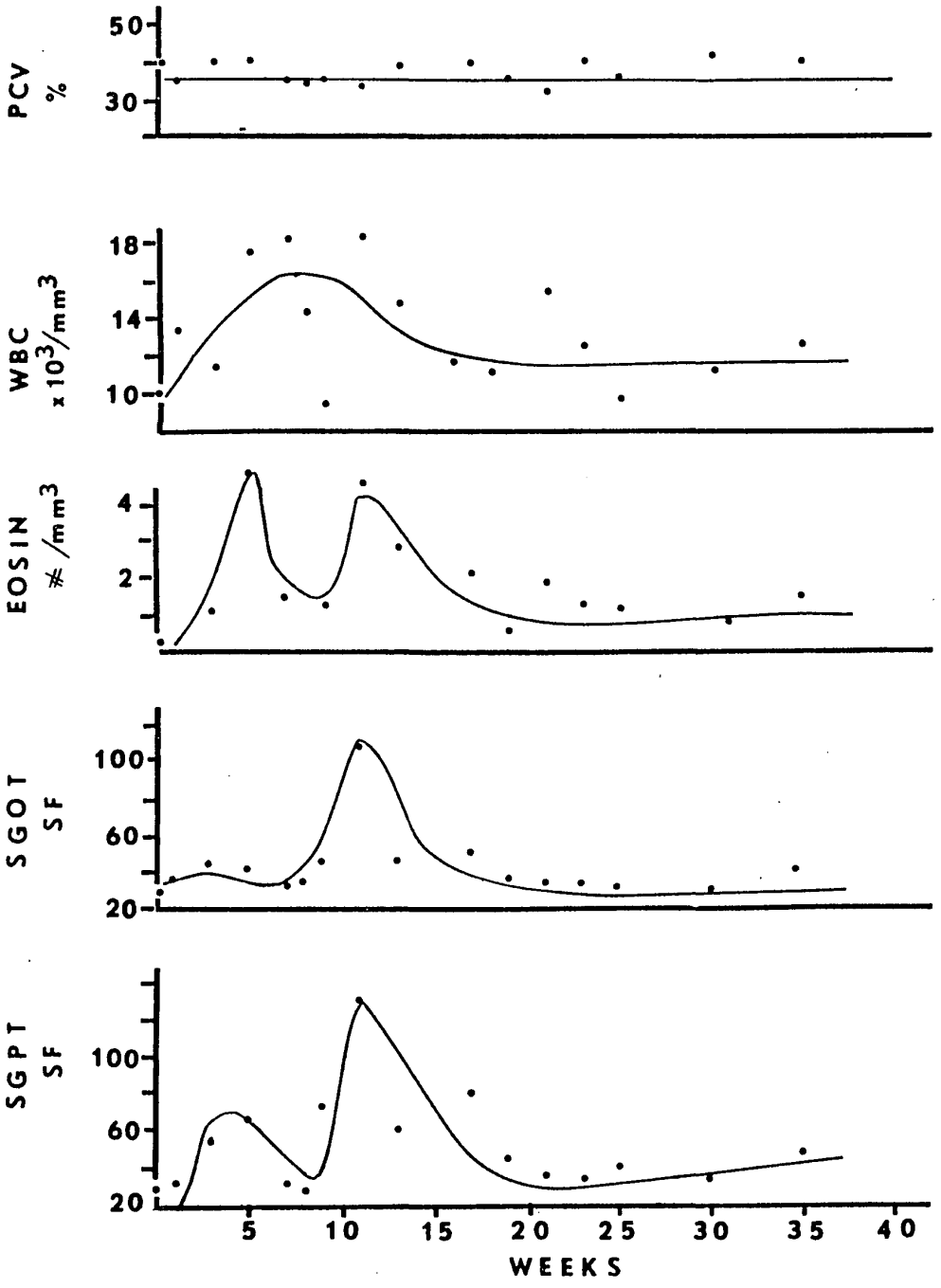


FIGURE 18

HEMATOLOGICAL VALUES FOR CAT No. 28
GROUP C - FEMALE

month and a half of experimental infection. The hematological profile of cat #29 is shown in Figure 19. Serum transaminase enzyme levels peaked during week 12. Eosinophilia reached maximal levels during weeks 6 and 12, while slight leucocytosis was observed during week 8. Thus, 2 female cats of equivalent weight and age, receiving the same infective dose, presented different hematological responses. Thus, each animal should be considered as an individual case report. For this reason all data for each animal has been included in the Appendix. However, for simplicity, average group values are presented in the result section. The general changes which occur during the course of platynosomiasis can be deduced from the group averages. Statistical ranges have not been included because each experimental group contained small numbers of animals and these animals presented a wide range of values for any single parameter monitored.

3. Examination of Fecal Samples for the Presence or Absence of Cat Liver Fluke Ova

All fecal samples collected 5--7 weeks after infection from cats in Group B and C were positive for Toxocara cati ova. The number of ova passed was extremely small. Cats in Group A remained Toxocara negative. As a result of this finding, all cats received 2 oral treatments of piperazine (2 cc/kg body weight) weeks 13--14 post-infection. All cats treated, except two, were toxocara negative 4 months after the initial platynosomum infection. The two Toxocara positive cats were not treated further but used as a comparative study to better evaluate the role of this second parasite

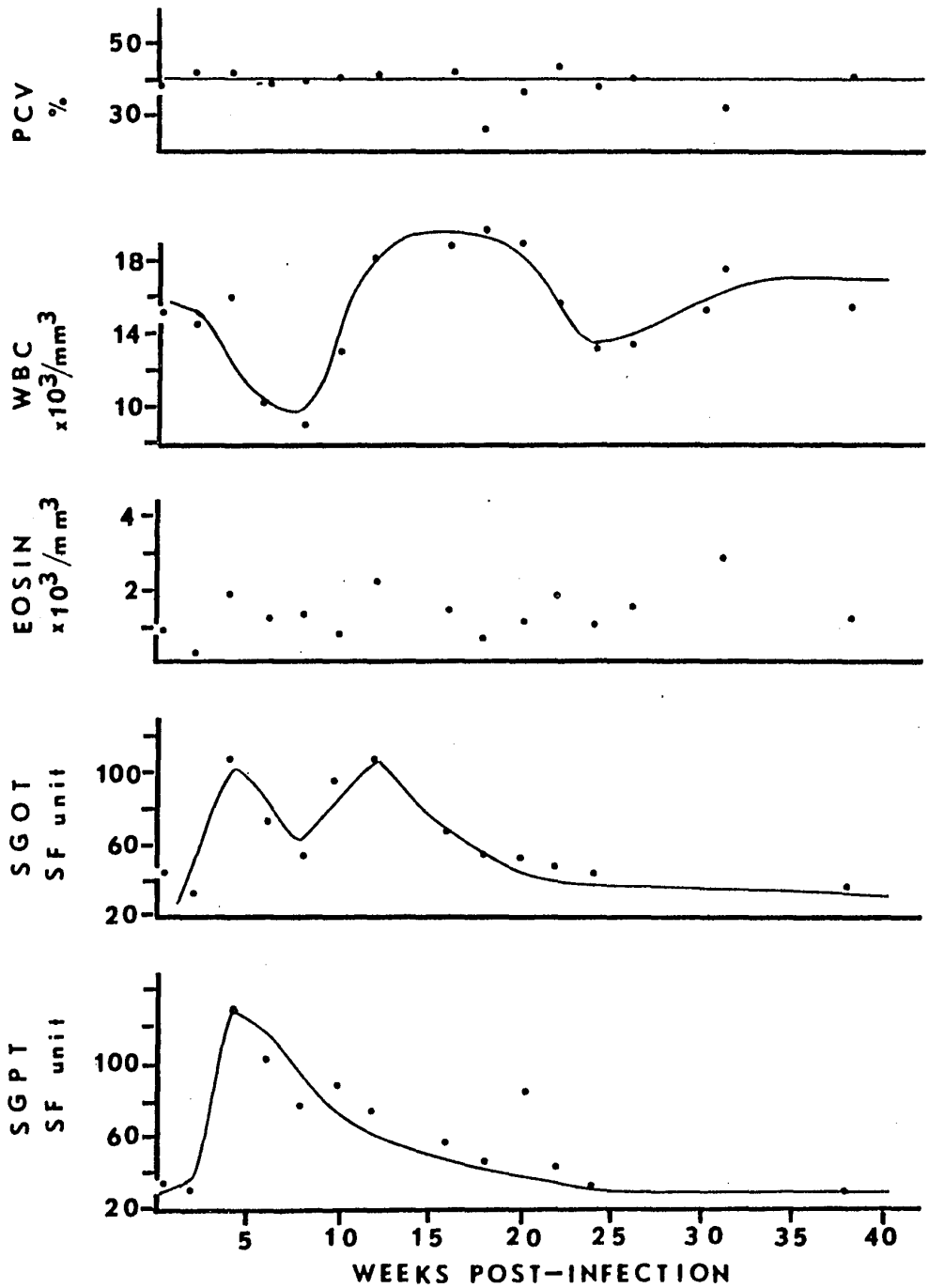


FIGURE 19

HEMATOLOGICAL VALUES FOR CAT NO. 29
GROUP C – FEMALE

in modifying the response to P. concinnum infection.

Immature P. concinnum ova were detected in fecal samples as early as 8 weeks post-infection. All animals in Groups B and C were diagnosed Platynosomum-positive by fecal analysis 12 weeks after infection (arrow represents this event in Figure 20). All Group A animals remained free of Platynosomum ova. Mature ova were present in feces 12 weeks after infection.

Figure 20 shows the average number of ova detected per gram of feces using the formalin-ether concentration technic. During the first 8 months following infection, Group C animals produced 2.7 times more ova than Group B animals. Group C cats received an infective dose 8.2 times Group B cats. Note that the number of ova detected in the feces decreased during the course of infection as seen in Figure 20.

4. Necropsy Results

Selected cats were euthanised and necropsied 1, 2, 3, 3.5, 6, 16, 24 and 36 weeks after infection. The gross pathologic observation for each animal is summarized below.

Week 1 Post-infection:

Group B male cat (#9) orally exposed to 328 infective stages, at necropsy presented a liver that was normal in size with slight mottling throughout. The edges were sharp except around the gall bladder. The bile ducts were not enlarged. The thin walled gall bladder was noticable distended and contained abundant bile that was yellow and fluid. The mesenteric lymph nodes, kidneys, and other

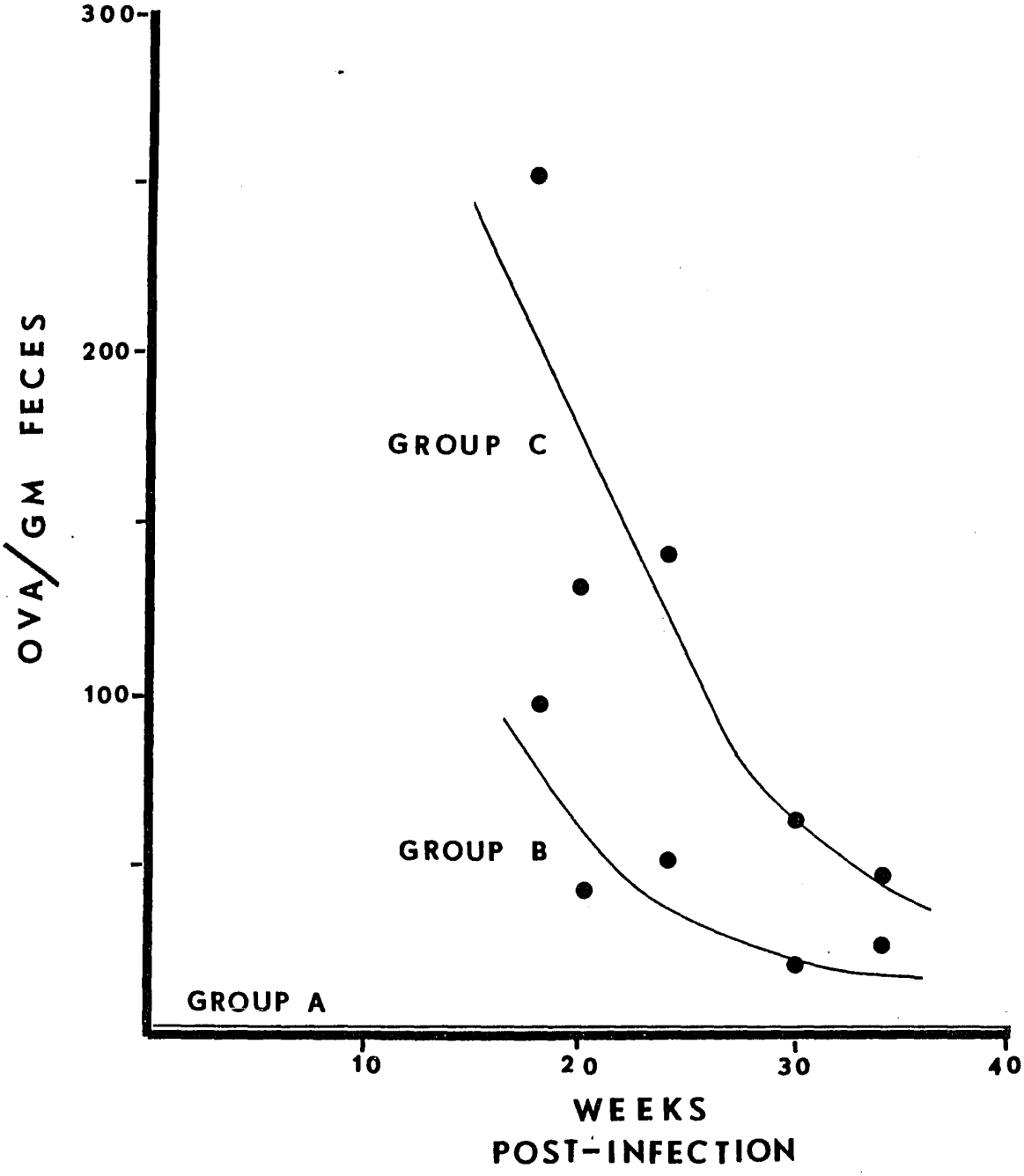


FIGURE 20

NUMBER OF OVA/GRAM FECES DETECTED BY FORMALIN-ETHER CONCENTRATION METHOD DURING P. CONCINNUM INFECTION

major thoracic and abdominal organs were normal.

Seven days after infection 303 immature flukes representing 91.7% of the original infective dose were recovered from the biliary tract.

Week 2 Post-infection:

Group B female cat (#17) orally exposed to 328 infective stages, showed at necropsy that the liver was normal but with a slight mottled surface. The gall bladder was distended with bile and slightly enlarged. The bile ducts were not noticeably enlarged. The mesenteric lymph nodes at the ileo--caecal junction were enlarged. All other thoracic and abdominal organs were normal.

302 immature flukes (91.7% of the initial infective dose) were recovered from the biliary tract.

Week 3 Post-infection:

Group B male cat (#10) had been exposed to 240 infective stages. At necropsy the liver was normal; no major changes were seen in the bile ducts or gall bladder. The mesenteric lymph nodes were not enlarged. No pathology attributable to platynosomiasis was noted.

The cat however presented signs of hemorrhagic cystitis due to urethral obstruction, and the kidneys were enlarged with slight venous congestion.

43 flukes, representing only 17.5% of the infective stages given were recovered 21 days after infection.

Week 3.5 Post-infection:

Group B male cat (#11) had been orally exposed to 328 infective stages. At necropsy the liver was mottled and pale with

rounded edges throughout. The bile ducts were normal. The gall bladder was enlarged and bilobed in appearance with thin, transparent walls. Pale yellow-green bile was present in the distended gall bladder.

All other organs were normal. 264 flukes, or 80.5% of the original infective stages were recovered 31 days after infection.

Week 6 Post-infection:

Group B male cat (#12) had been exposed to 240 infective stages. The surface of the liver showed no changes in gross pathology. The edges were sharp and the biopsy site was well healed. A "second" gall bladder was present. This smaller lobe of the gall bladder appeared as an outpocketing of the cystic duct (Figure 21). The blood vessels of the gall bladder were noticeably engorged. A small volume of yellow, transparent bile was present. An enlarged lymph node at the ileo-caecal junction was present. The most proximal bile ducts of all four lobes of the liver were enlarged.

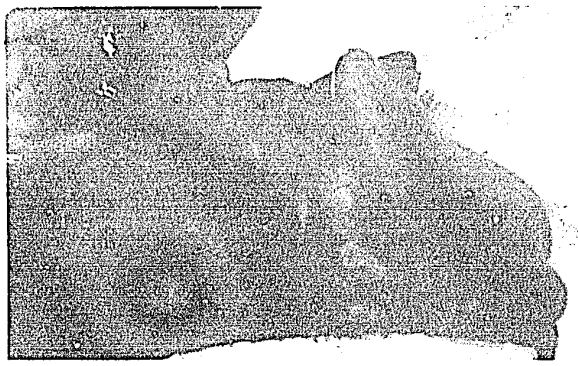
A recent bout of cystitis was evident from the pathologic changes in the epithelium of the urinary bladder. Some fibrosis was present in the kidneys.

135 flukes, representing 71.1% of the infective stages given, were recovered 42 days after infection.

Week 16 Post-Infection:

Group C female cat (#26) was given a dose of 1953-infective stages. The liver of this animal showed some gross pathology. It was of normal coloration but with rounded edges and slight enlargement. The surface had a mottled in appearance and a small, white necrotic

Liver from Group B male cat No. 12, 6 weeks post-infection. Note enlargement of cystic duct giving the appearance of a "second" gall bladder.



Note enlarged bile ducts, 4 months post-infection. From Group C female cat No. 26.

1 cm

FIGURE 21

LIVER PATHOLOGIC CHANGES DURING EXPERIMENTAL PLATYNOSOMIASIS

area was noted on the surface of the left lobe. The bile ducts were notably enlarged throughout the entire liver (Figure 21). The gall bladder was bilobed and contained clear, thin bile within its almost transparent walls. P. concinnum ova were found upon microscopic examination of the bile.

The hepatic lymph node was greatly enlarged measuring 12 mm in diameter and contributed to constriction of the common duct. Other mesenteric lymph nodes were slightly enlarged. The kidneys were small. The cortex showed intense yellow-green staining; little coloration was noted in the medulla. This cat had an elevated icterus index 9 weeks prior to necropsy but, when alive, presented no clinical signs of jaundice such as yellow skin or mucosa.

All other organs were normal in appearance. 829 flukes, or 42.5% of the infective dose given were recovered 110 days after infection.

Week 24 Post-infection:

Group C female cat (#27) had been orally exposed to 1978 infective stages. At necropsy the surface of the liver was normal and it was not enlarged as in the previous cats. Bile was of a normal consistency and cat liver fluke ova were present. The bile ducts were enlarged and hardened throughout the liver.

Enlarged mesenteric lymph nodes were noted especially at the ileo-caecal junction. A possible statement about lymph node involvement is not possible since the cat had symptoms of enteritis (of unknown etiology) when selected for necropsy.

All other organs were normal. 203 mature flukes, representing

10.3% of the infective stages given were recovered 187 days after infection.

Week 36 Post-infection:

Four cats were euthanised and necropsied at this time.

1. Group B male cat (#13) had been exposed to 240 infective stages. The liver of this animal was swollen with rounded edges; but the surface was normal. The gall bladder was distended with clear, yellow bile. P. concinnum ova were present in the bile. Bile ducts were enlarged throughout the entire liver.

Other organs were normal. 127 mature flukes, or 52.9% of the infective dose given were recovered.

2. Group B female cat (#18) had been given a dose of 240 infective stages. The exterior appearance of the liver of this animal was completely normal, but when cut it was immediately evident that the bile ducts were noticeably enlarged. The gall bladder was distended with thick, dark bile and upon microscopic examination P. concinnum ova were found.

No other pathologic changes attributable to platynosomiasis were present.

This cat had shown signs of continual estrum for approximately 5 months prior to necropsy. Cystic ovaries, each with multiple corpora lutea were found. The uterus was enlarged and oozed purulent material when cut, indicative of pyometra.

118 mature flukes, or 47.2% of the infective dose given were recovered 252 days after infection.

3. Group C male cat (#22) had been given a dose of 1978

infective stages. At necropsy, major pathologic changes were noted on the mottled, brown surface of the enlarged liver. Multiple grey-white foci (2 mm in diameter) were present on the ventral margin of the right hepatic lobe. All major bile ducts were greatly enlarged.

The gall bladder was approximately 10 times the normal size (1.5--2.0 cm in diameter) and formed a tortuous spiral. The cystic duct emptying into the duodenum had an enlarged diameter (2.5 mm). The gall bladder was filled with light green bile, ova of P. concinnum, and adult flukes.

Hepatic and mesenteric lymph nodes were enlarged and hemorrhagic. A reddish zone between the medulla and cortex of the kidneys was noted. All other organs were normal. 457 flukes, or 23.1% of the infective dose given were recovered.

4. Group C male cat (#23) had been exposed to 1978 infective stages. At necropsy the liver of this cat presented the most pronounced pathologic changes thus far encountered. The surface of the liver showed "tract marks" indicative of pronounced underlying fibrosis and had a cobblestone appearance. The parenchyma was extremely friable. The outline of enlarged and hardened bile ducts influenced the surface texture of the liver. Adhesions were seen in the biopsy site. The gall bladder was distended with dark, viscous bile. Hemorrhagic areas were present on the surface of the gall bladder.

Three hepatic lymph nodes were enlarged but not hemorrhagic. The left kidney was small and presented a scar on the surface denoting trauma prior to the experiment. The right kidney was normal

as were all other organs.

1,196 flukes or 60.5% of the infective dose given were recovered in this animal.

A summary of some of the major changes in the liver is shown in Figure 22.

5. The Number of Flukes Recovered at Necropsy

The number of flukes recovered at necropsy was determined at various times during the course of infection. The fluke recovery rate expressed as percentage of the infective dose orally administered is shown in Figure 23. This recovery percentage diminished during the first 6 months of experimental infection, but was higher 9 months post-infection. These observations support the thesis that a self-cure mechanism probably is not involved but rather that the percentile recovered with time reflected individual susceptibility to the initial infectious material. An average 53.7% of the infective dose was recovered from all cats but the range was very wide (10.3% to 91.7%).

6. Distribution of Flukes within the Biliary Tract

The distribution of flukes within the individual lobes of the liver appeared to be random. The data presented in Table 4 show no predominance of flukes within the bile ducts of any one of the four lobes during the course of the disease. Examination of the liver at necropsy revealed an average of 4.2 flukes/gram median lobe hepatic, 4.9 flukes/gram caudate lobe, 3.3 flukes/gram left lobe and 3.0 flukes/gram right lobe. In general, however, the number of

Time (Weeks) Post-Infection	Enlarged Gall Bladder	Bilobed Gall Bladder	Enlarged Mesenteric and/or Hepatic Lymph Nodes	Enlarged Bile Ducts	Paranchymal Damage
0	-	-	-	-	-
1	+	-	-	-	-
2	+	-	+	-	-
3	-	-	-	-	-
3.5	+	+	-	-	-
6	+	+	+	+	-
16	+	+	+	+	-
24	±	-		-	-
36	+	-	-	+	-
	+	-	-	+	-
	+	-	+	+	-
	+	-	+	+	+

FIGURE 22

PATHOLOGIC FINDINGS RECORDED AT NECROPSY

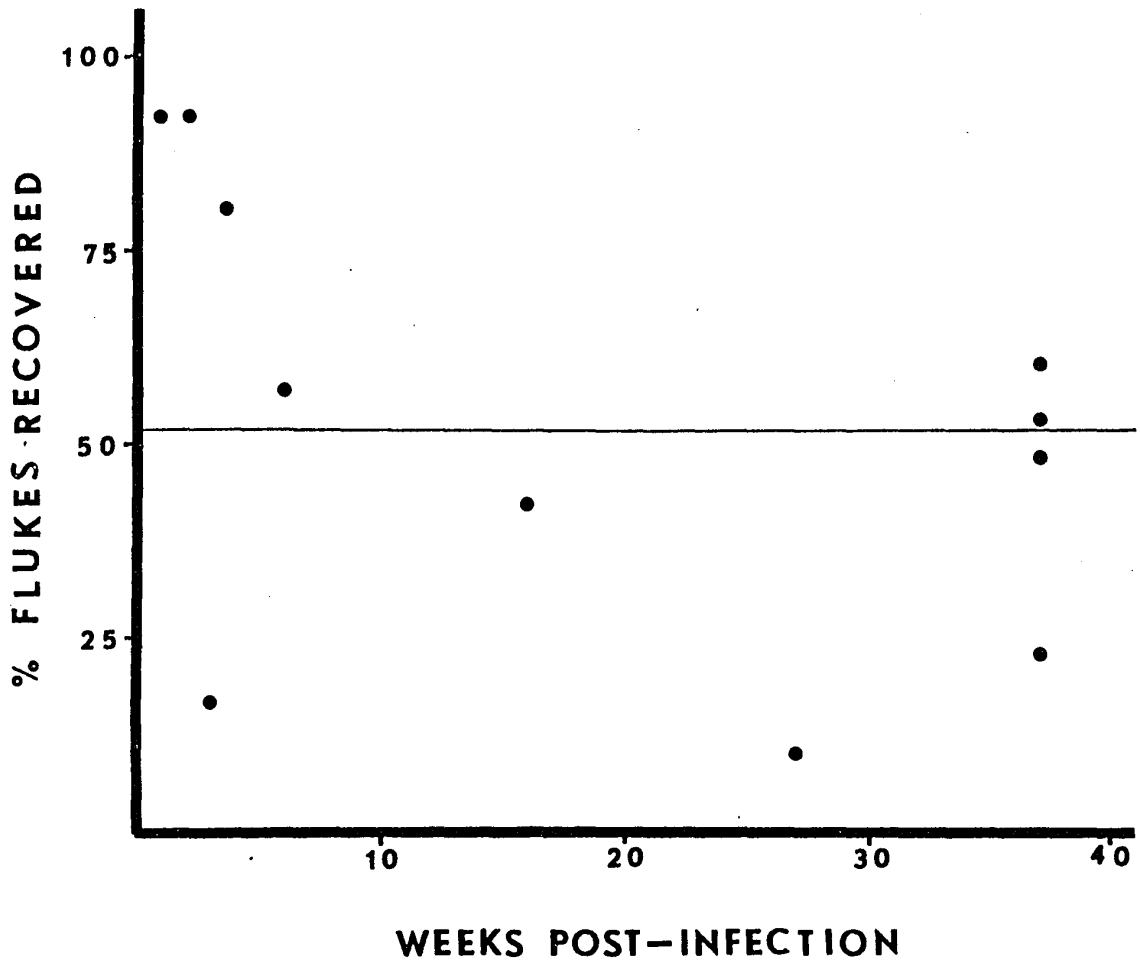


FIGURE 23

PERCENTAGE OF FLUKES RECOVERED AT NECROPSY
DURING THE COURSE OF INFECTION

TABLE 4

DISTRIBUTION OF FLUKES WITHIN THE LOBES OF THE LIVER

WEEKS AFTER INFECT.	% FLUKES/LOBE				GALL BLADDER	NUMBER FLUKES/GM			
	MEDIAN	RIGHT	CAUDATE	LEFT		MEDIAN	RIGHT	CAUDATE	LEFT
1	22.6	33.9	15.6	27.9	0	4.1	3.5	5.4	3.4
2	22.7	29.5	18.9	28.8	0	4.6	3.3	6.7	4.1
3	7.0	27.9	10.6	41.9	4.7	0.2	0.4	0.7	0.6
3.5	44.3	20.5	4.9	39.5	0.8	6.8	1.7	1.5	3.0
6	26.7	40.0	12.4	22.9	0	1.8	2.0	1.0	1.0
16	29.3	19.4	6.5	36.8	8.0	10.3	5.4	6.4	1.8
24	11.8	34.6	8.9	38.9	15.8	1.5	2.3	8.3	1.1
36	17.8	31.4	7.6	31.4	11.9	1.8	0.9	1.1	1.6
36	15.7	27.6	15.7	31.5	9.4	0.9	0.8	1.7	1.2
36	5.3	17.1	6.3	25.6	45.7	1.5	3.9	2.5	2.2
36	19.0	22.1	13.7	41.7	3.5	12.6	8.4	18.2	16.6

flukes recovered from the gall bladder increased with time.

7. Histopathologic Changes during Experimental Infection

Microscopic examination of hepatic tissue biopsied prior to experimental infection were normal. Liver parenchymal cells with ovoid nuclei, prominent nucleoli and granular cytoplasm surrounded small bile ducts. Few bile ducts were present in the biopsy material, but those present were lined with typical cuboidal ductal cells (Figure 24). A few leucocytes were noted in the sinusoidal spaces and within the blood vessels.

The histologic changes noted in hepatic tissue from experimental platynosomiasis are summarized below.

Week 1 Post-infection:

No histologic changes were noted (Figure 24).

Week 2 Post-infection:

15 days post-infection liver flukes could be demonstrated in the bile ducts; but only minimal histologic changes were evident. The ductal cells maintained a cuboidal appearance despite the presence within the lumen of the fluke. Connective tissue began to surround some bile ducts. A slight leucocyte infiltration was noted around a few major ducts.

Week 3.5 Post-infection:

26 days post-infection definite, but still relatively minor, alterations of the liver architecture were evident (Figure 25). Flukes within major bile ducts were attached to epithelial folds of the duct wall. Some ductal cells were columnar in shape. Pleats of

Minor bile duct in biopsied material.

A portion of the portal triad 7 days post-infection.

FIGURE 24

HISTOPATHOLOGIC SECTIONS OF HEPATIC TISSUE
BEFORE AND SEVEN DAYS AFTER P. CONCINNUM INFECTION

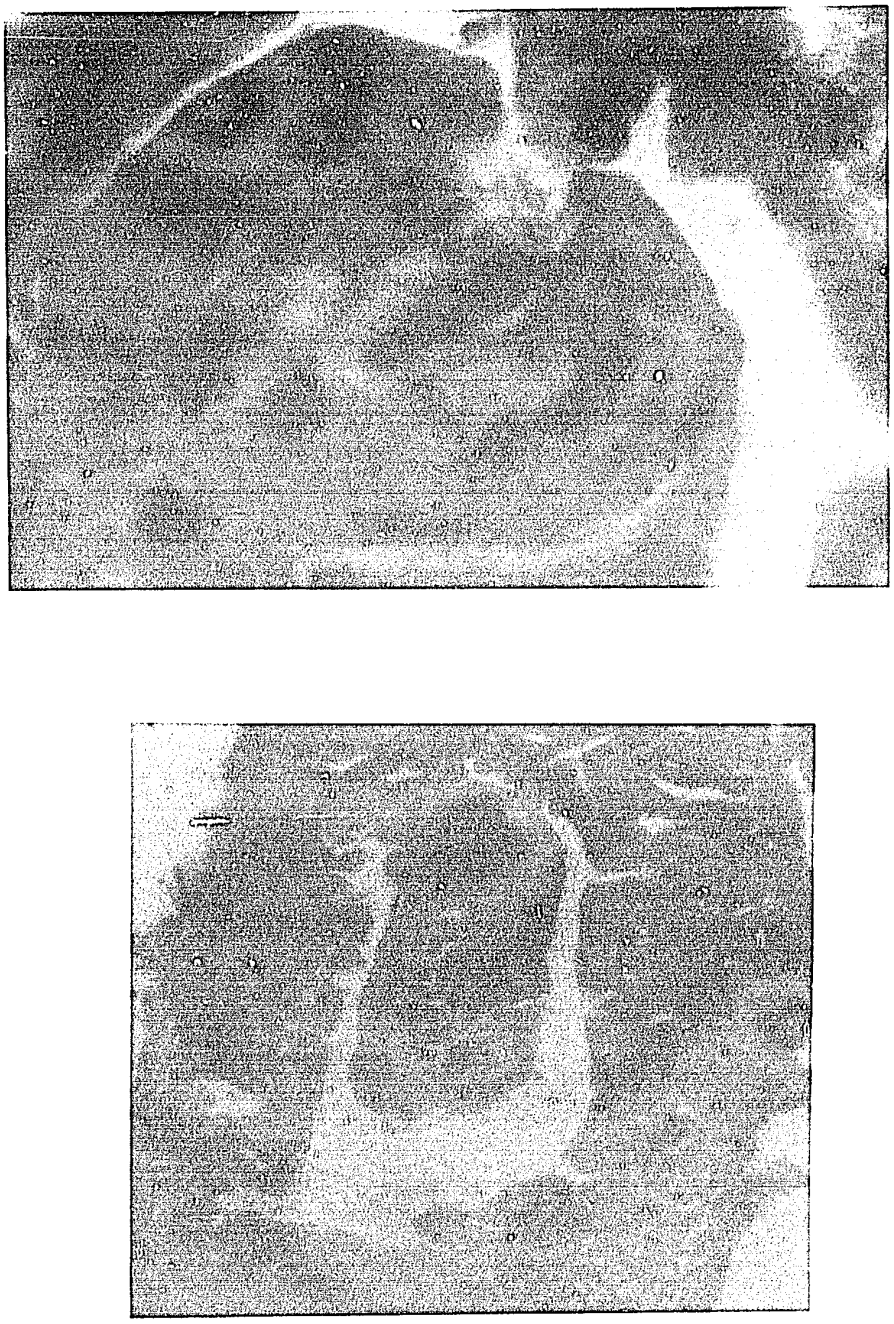


FIGURE 25

HISTOPATHOLOGICAL SECTIONS OF P. CONCINNUM WITHIN A MAJOR BILE DUCT 26 DAYS POST-INFECTION

duct tissue evaginated from the bile duct lumen indicating incipient secondary bile duct formation (Figure 26). Infiltration of lymphocytes, eosinophils, and polymorphonuclear leucocytes was now pronounced.

Week 6 Post-infection:

Forty-two days post-infection the histologic findings were markedly abnormal. Massive leucocyte infiltration surrounded many of the bile ducts (Figure 27). Many lymphocytes were present with "streaks" of eosinophils coursing through these area. The bile duct epithelial lining had begun to thicken, suggestive of cellular stratification in some areas (Figure 28).

Week 16 Post-infection:

One hundred ten days post-infection the hepatic changes were similar to those at 6 weeks but more extensive. Severe adenomatous hyperplasia of bile duct epithelium with periductual inflammation was present. The abundant glandular acini (Figure 29) were supported by fairly delicate connective tissue stroma. The acini arranged radially about the lumen of the duct were lined by a single layer of columnar epithelial cells. Fibrotic connective tissue surrounded several peribiliary plexus.

Week 24 Post-infection:

Relatively few parenchymal changes were noted. Adenomatous hyperplasia, leucocytic infiltration and mild fibrosis was present around major bile ducts. However, many minor ducts remained unaltered.

Week 36 Post-infection:

An equal distribution of adenomatous and connective tissue was present in the biliary tract 257 days after infection. A chronic

Some ductal cells were columnar in shape. Pleats of bile duct tissue evaginated from the bile duct lumen indicating incipient secondary bile duct formation.

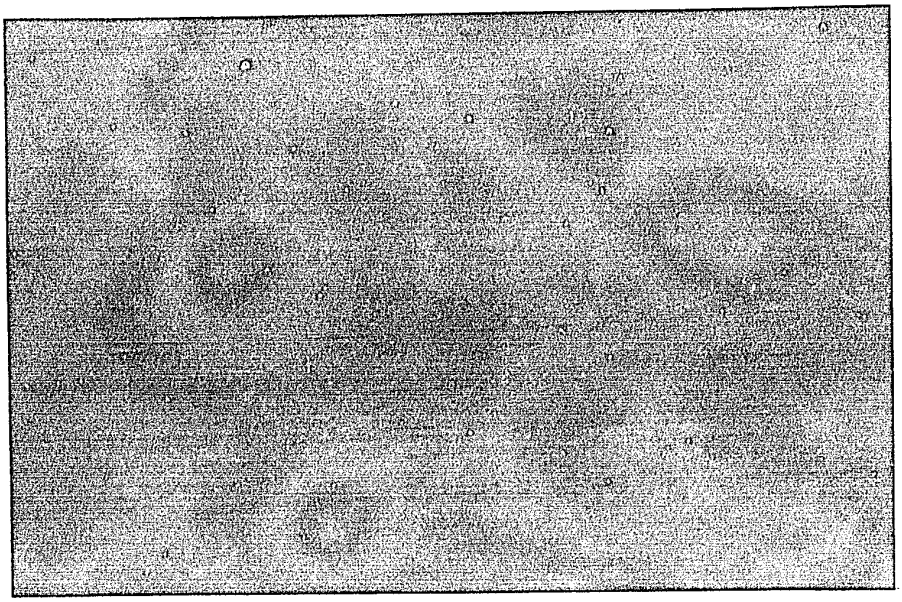
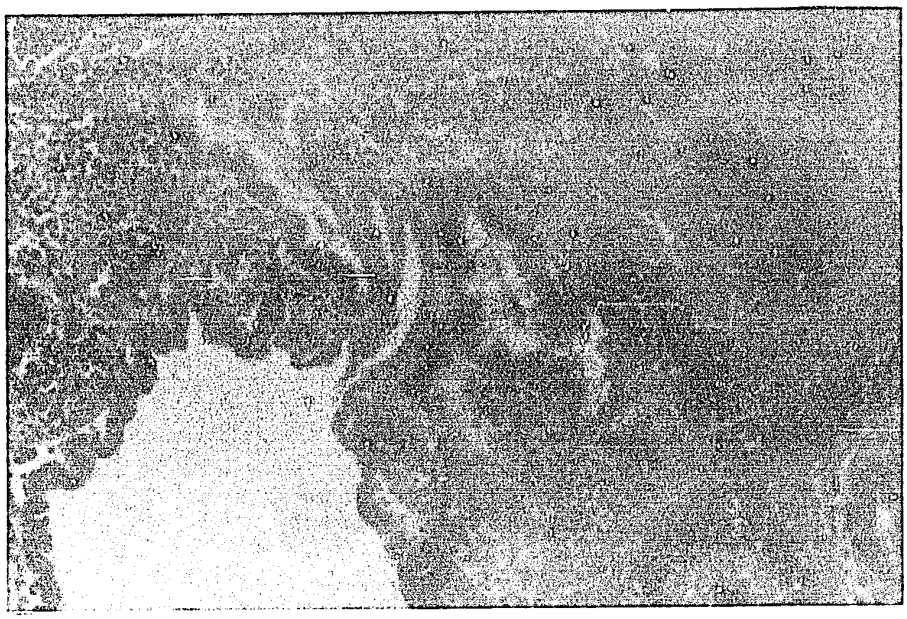


FIGURE 26

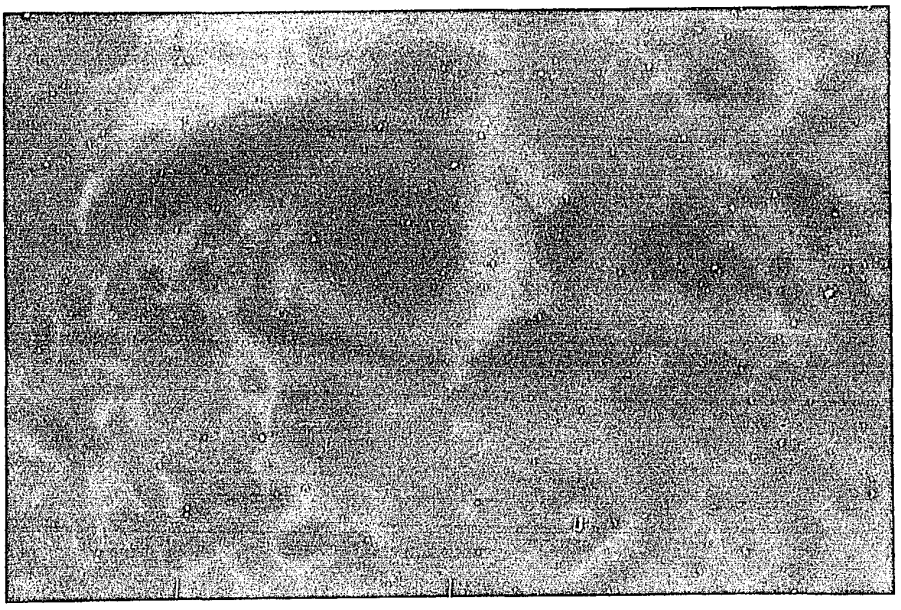
HISTOPATHOLOGIC SECTIONS OF LIVER 26 DAYS POST-INFECTION



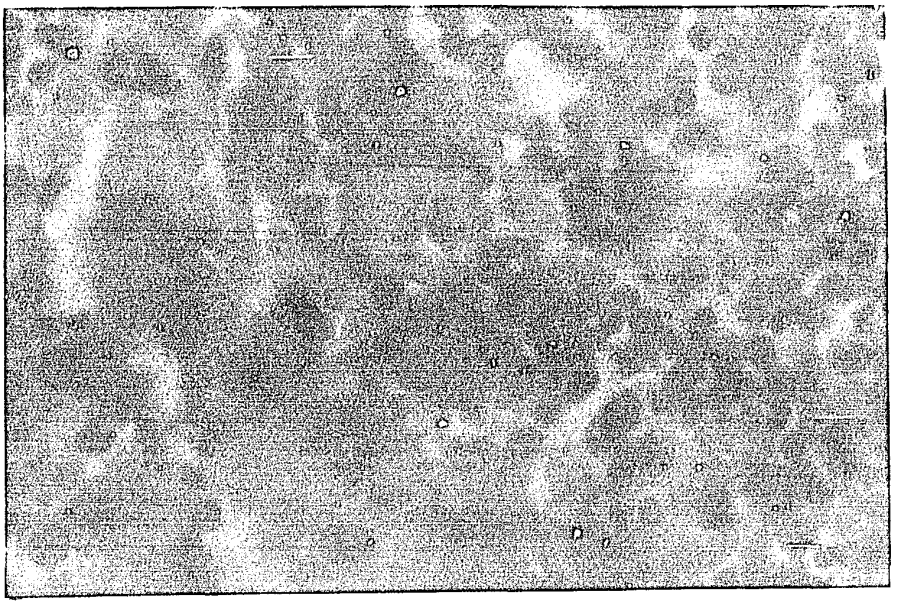
Massive leucocyte infiltration
surround many of the bile ducts.

FIGURE 27

HISTOPATHOLOGIC SECTIONS OF HEPATIC TISSUE
6 WEEKS POST-INFECTION



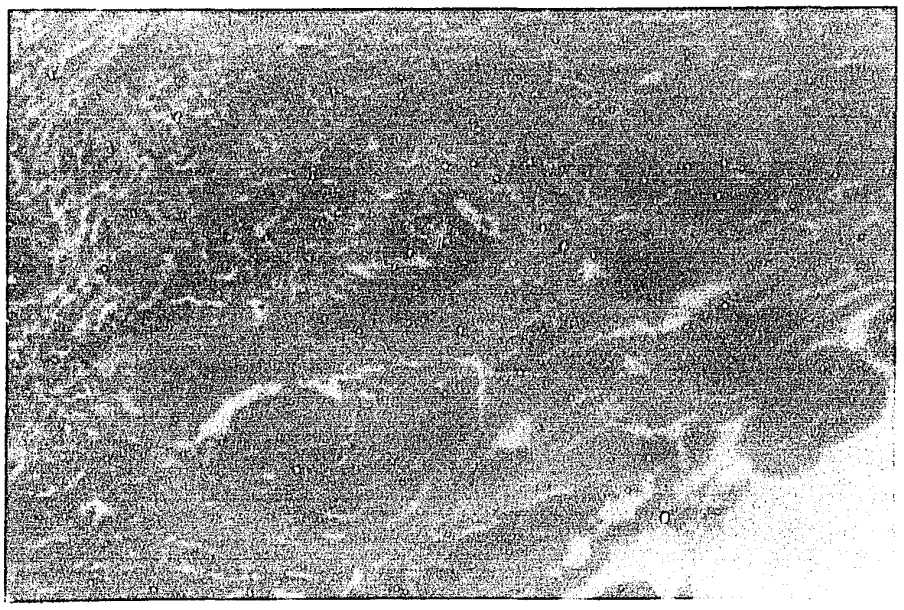
The bile duct epithelial lining had begun to thicken, suggestive of cellular stratification in some areas.



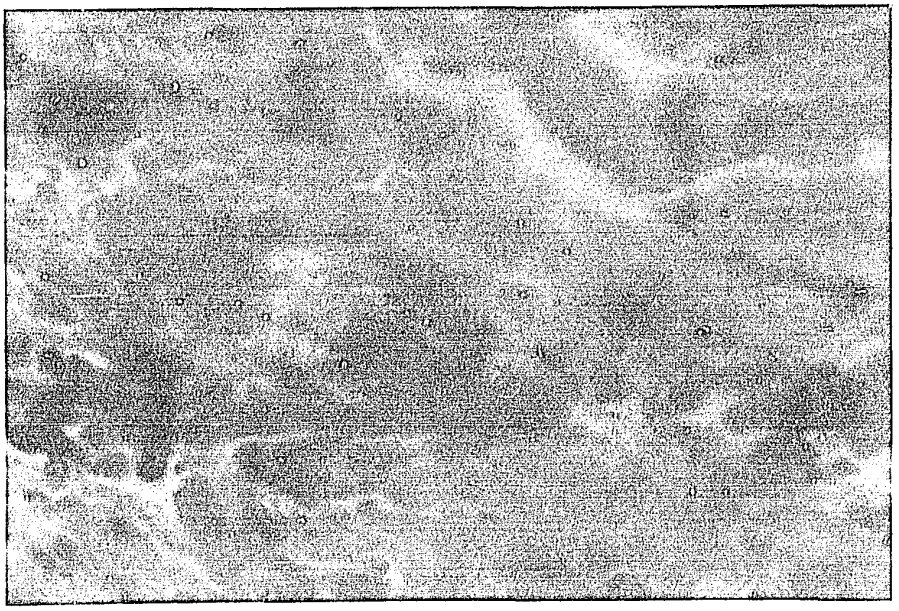
Massive cellular infiltration of lymphocytes, polymorphonuclear leucocytes, and eosinophils surround the damaged bile ducts.

FIGURE 28

HISTOPATHOLOGIC SECTIONS OF HEPATIC TISSUE
6 WEEKS POST-INFECTION



Severe adenomatous hyperplasia of bile duct epithelium with periductal inflammation was present.



Glandular acini were supported by delicate connective tissue stroma.

FIGURE 29

HISTOPATHOLOGIC SECTIONS OF HEPATIC TISSUE
4 MONTHS POST-*P. CONCINNUM* INFECTION

inflammatory reaction was present around the dilated bile ducts with many eosinophils present. The biliary pathology was the same in Group B and C animals indicating no correlation with the number of parasites present.

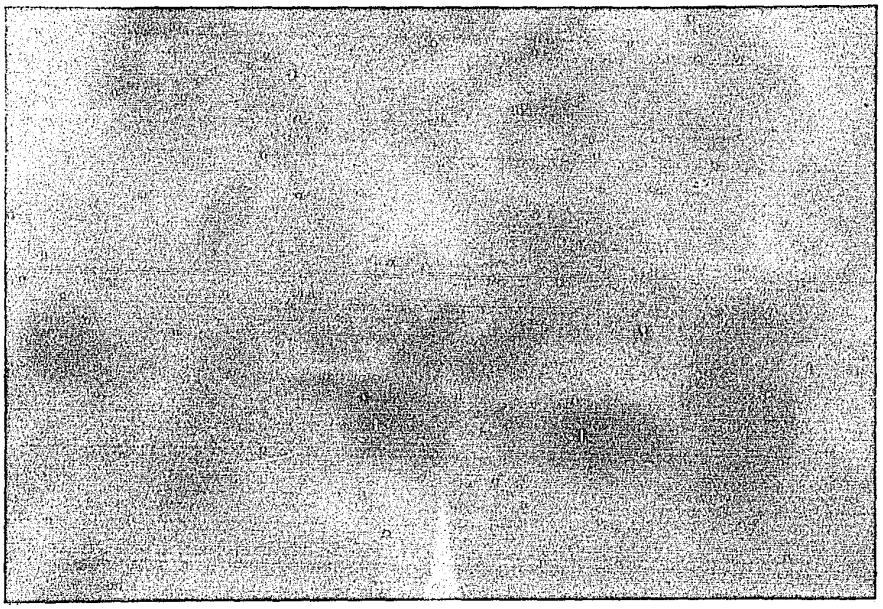
Examination showed no microscopic evidence of multilobular hepatic cirrhosis initiated by P. concinnum. Multiple scars however were frequently found between dilated bile ducts in the late stages of infection. These scars were composed of proliferated bile ducts and connective tissue and lacked parenchymal cells, presented dense mononuclear-cell infiltration.

Cholestasis, as evidenced by "bile plugs" in bile canaliculi was present in cat #23. In this case the stasis probably was localized or intermittent since histologic sections from other lobes of its liver did not show these bile plugs (Figure 30).

8. Growth Rate and Developmental Stages of Platynosomum concinnum

The infective stage found in the liver of the toad (Bufo marinus) resembles the cercaria which emerges from the snail; but is larger in size (Sinclair, 1975). This stage can be pleomorphic (Plate 1) and extremely active. When extended, the infective stage may be 1 mm in length; when contracted maximally, 500 μ in length. The average size of this stage at rest is estimated as 750--800 μ 150--200 μ. This stage is considered to represent the metacercarial stage in the life cycle (Maldonado, 1945). Two suckers are present as is a large excretory bladder with excretory tubules extending to the anterior and posterior of the fluke (Figure 31). A short esophagus

Zones of glandular acini, cellular infiltration and connective tissue surround parasitized bile ducts.



"Bile plugs" in bile canaliculi were present in cat No. 23.

FIGURE 30

HISTOPATHOLOGIC SECTIONS OF HEPATIC TISSUE
9 MONTHS POST-INFECTION

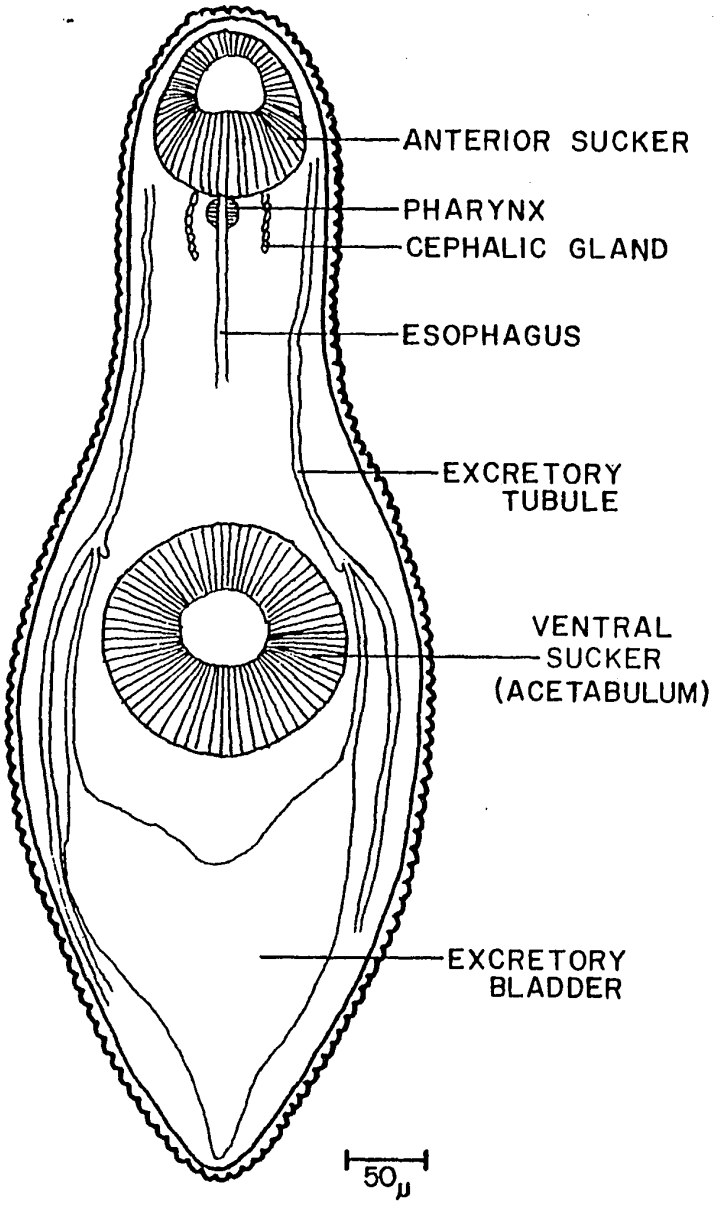


FIGURE 31

INFECTIVE STAGE ISOLATED FROM BILE DUCTS OF BUFO MARINUS

leads from a muscular pharynx. Unfortunately, the remainder of the digestive system could not be defined microscopically. A pair of cephalic glands are located on either side of the pharynx. The pattern of flame cells has not been determined. An excretory pore is present at the distal end of the fluke. Gonadal tissue is completely absent in this stage. The outer cuticle is thick with microvilli present.

All stages recovered from toad livers had the general features described above; apparently the parasite does not develop beyond this stage in this amphibian. When fed to cats, this arrested stage begins to complete its development. The growth rate of the parasite within the cat is presented in Figure 32. Once ingested the juvenile fluke undergoes a change in size and proportions by becoming shorter and wider during the first 2--3 weeks. Thereafter the length to width ratio increases with the development of the parasite. Data in Figure 32 indicate the flukes were still increasing in length 9 months after oral administration to the feline.

No change in fluke surface area was noted during the first 2--3 weeks of infection. However, during the following 34 weeks an almost linear increase of surface area with time was noted.

The developmental sequence of P. concinnum is described below.

Days 1--14 Post-infection: Figure 33

The juvenile flukes 1--2 weeks post-infection have the same morphological features as the infective stage. As seen in Figure 32 the proportions of the parasite change. Plate 2 depicts the special features possessed by both the infective stage and the juvenile fluke.

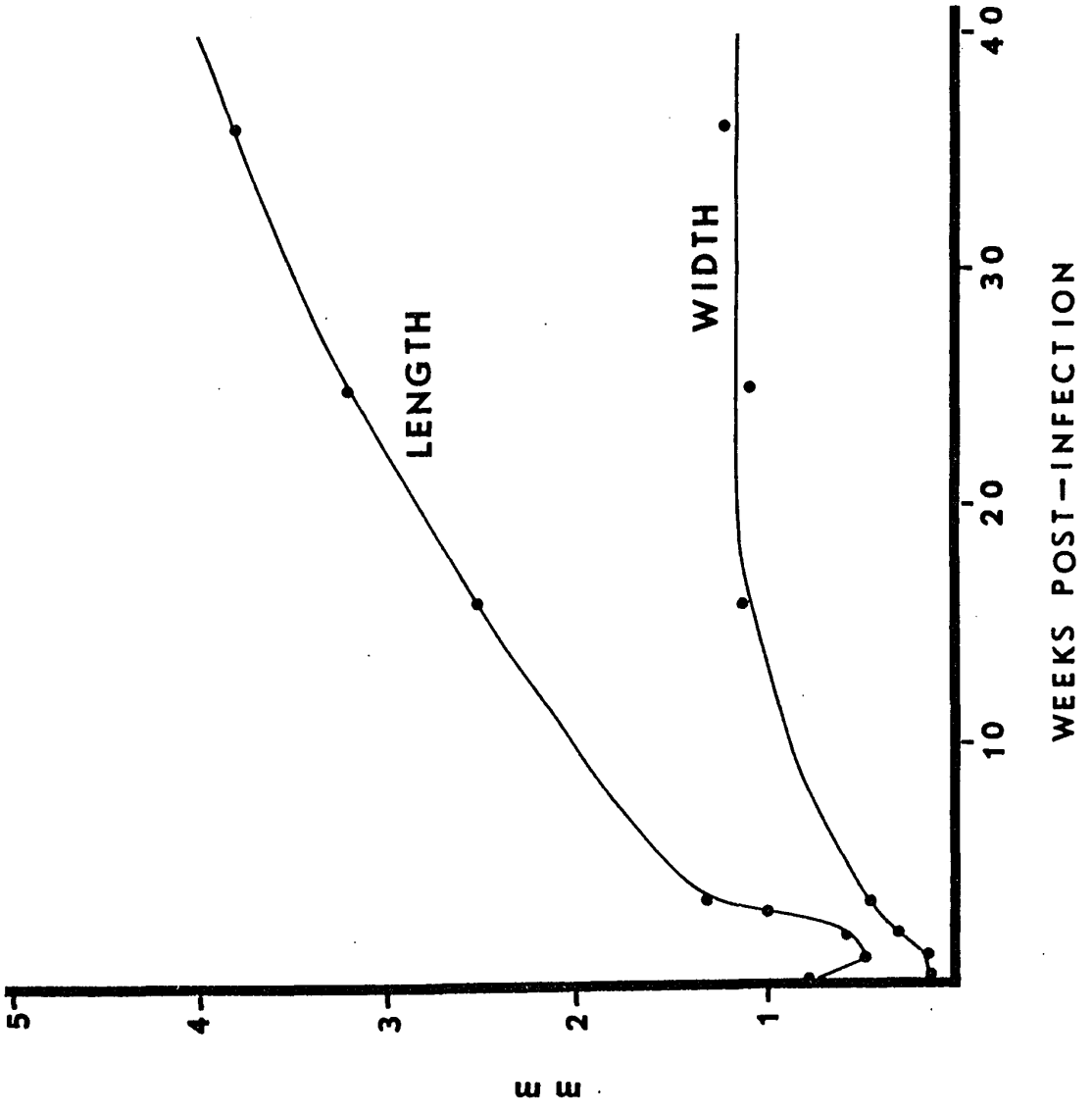


FIGURE 32

GROWTH RATE OF P. CONCINNUM IN THE CAT

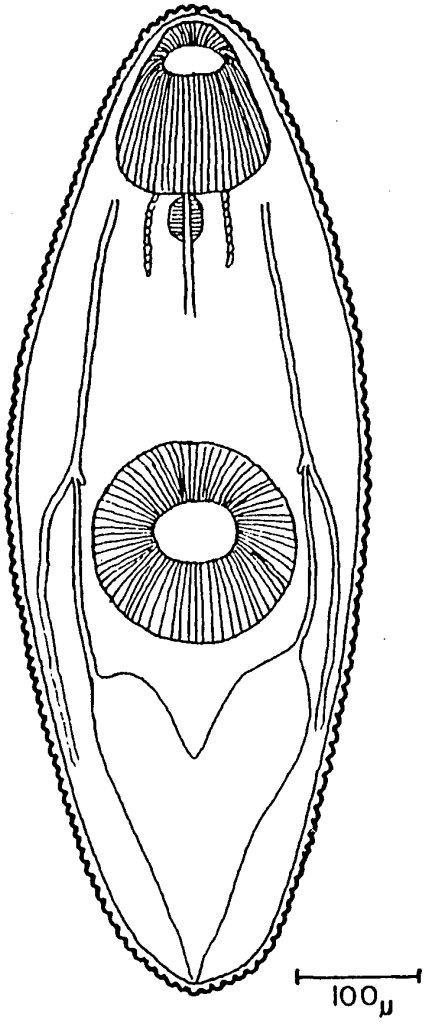


FIGURE 33

THE DEVELOPMENTAL STAGE OF P. CONCINNUM
PRESENT IN THE LIVER OF CATS 14 DAYS POST-INFECTION
(NOTE SCALE CHANGE)

Days 19--21 Post-infection: Figure 34

By 19 days the bifurcated digestive caeca course unbranched along either side of the fluke. The oviduct begins to form in a few flukes, Plate 3. By 21 days rudimentary testes containing large spermatocytes form just behind the acetabulum. All previously described structures have been retained.

Day 26 Post-infection: Figure 35

Further development of the fluke is marked by the addition of vitelline material in a few developing flukes.

Day 42 Post-infection: Figure 36

The testes and single ovary are well developed in approximately 70% of the flukes recovered at necropsy at this time. The cirrus sac containing the seminal vesicle, prostrate gland and ejaculatory duct formed in a few parasites. The vas deferens branched anterior to the acetabulum and then each branch proceeded posteriorly to connect with the testes.

A seminal receptacle containing motile sperm was present in approximately half the flukes. It is located in the immediate vicinity of the ovary and joins to the oviduct anterior to the newly forming Mehlis gland. Immature developing ova are present in approximately 10% of the flukes. A mixture of immature ova and vitelline material was occasionally noted in the uterus. It appeared that these flukes were ready to pass reproductive products in an immature and incomplete form, Plate 5.

Day 64 Post-infection: Figure 37

The maturation process was essentially complete. The Mehlis

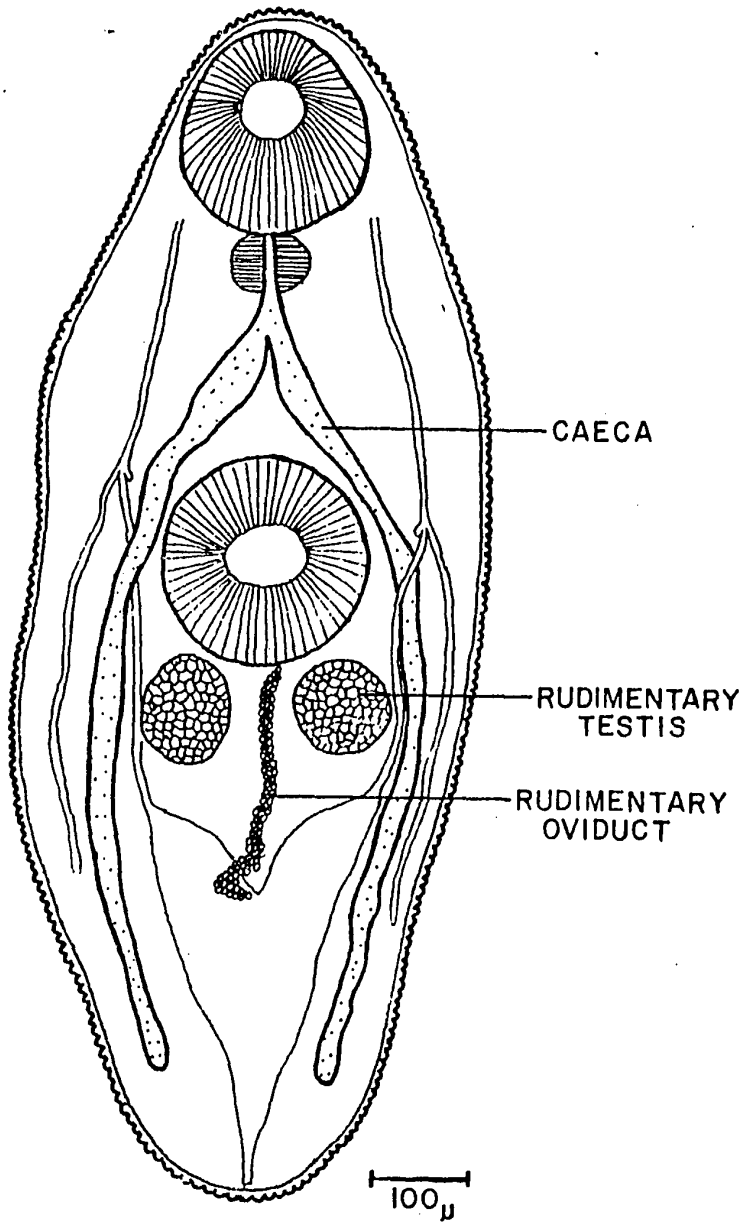


FIGURE 34

THE DEVELOPMENTAL STAGE OF P. CONCINNUM PRESENT IN THE LIVER OF CATS 19--21 DAYS POST INFECTION-OVIDUCT AND RUDIMENTARY TESTES ARE BEGINNING TO FORM

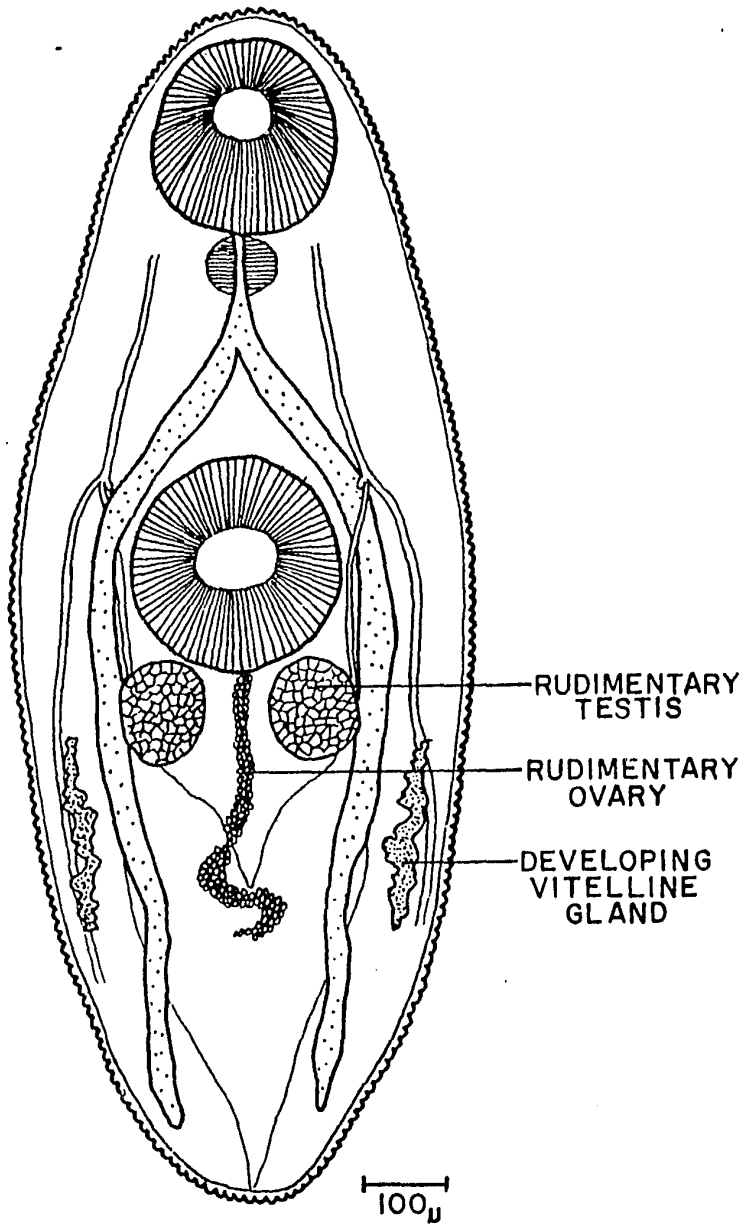


FIGURE 35

THE DEVELOPMENTAL STAGE OF P. CONCINNUM PRESENT IN THE LIVER OF CATS 26-DAYS POST INFECTION-VITELLINE GLANDS ARE BEGINNING TO FORM

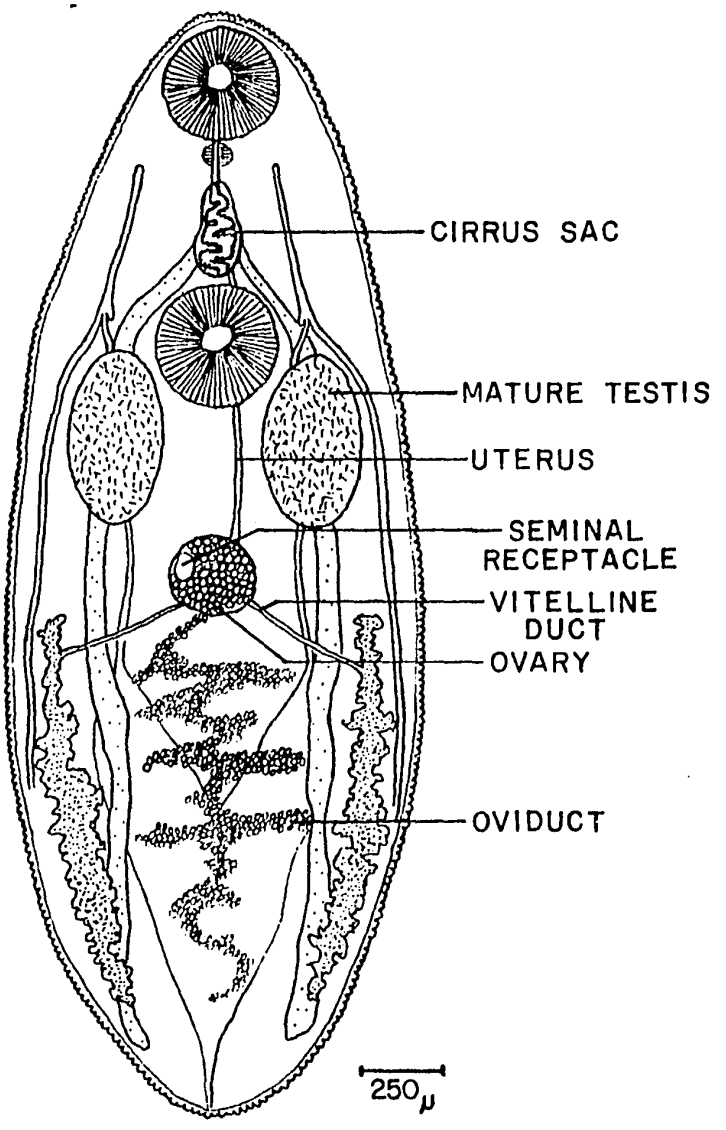


FIGURE 36

THE DEVELOPMENTAL STAGE OF P. CONCINNUM PRESENT IN THE LIVER OF CATS 42 DAYS POST-INFECTION

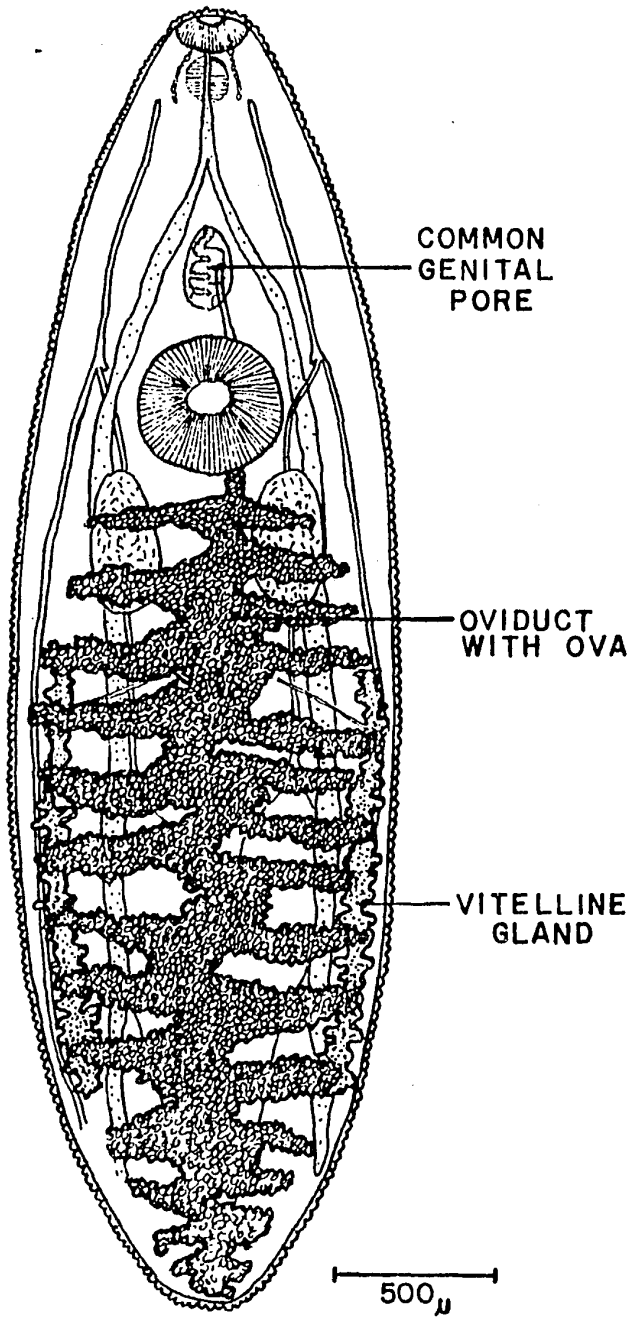


FIGURE 37

THE DEVELOPMENTAL STAGE OF P. CONCINNUM PRESENT IN THE LIVER OF CATS 64 DAYS POST-INFECTION

gland was completely developed and functional. The now branched oviduct was distended with thick-shelled ova in the process of embryonation. Mature embryonated ova, however, were not yet present (Plate 6). The male and female reproductive organs share a common gonadal pore.

9. Assessment of the Regeneration Capacity of Hepatic Tissue

An autoradiographic analysis was made on liver tissue collected during the course of experimental P. concinnum infection. The results are summarized in Figure 38. Only nuclei of hepatocytes were labeled (Figure 39); tritiated thymidine was not incorporated into ductal, Kuppfer, or connective tissue cells during the 90 minute incubation period. The number of labeled hepatocytes remained the same throughout the course of infection when compared with the initial biopsied material.

10. Results of Enzymatic Analysis of Hepatic Tissue in Experimentally Infected Cats

The levels of activity of several enzymes studied in the hepatic tissue of experimentally infected cats during the course of infection are graphed in Figures 40 and 41. A slight decrease in GPOT enzyme activity was recorded during the first four months of infection but returned to normal levels thereafter. The other transaminase enzymes, GPT, showed a minor depression during the early phase of infection but remained relatively stable. A general depression of peroxisomal oxidative enzyme activities was noted (DAAO, OHAO, uricase-Figure 41). Catalase enzymatic activity was initially depressed but

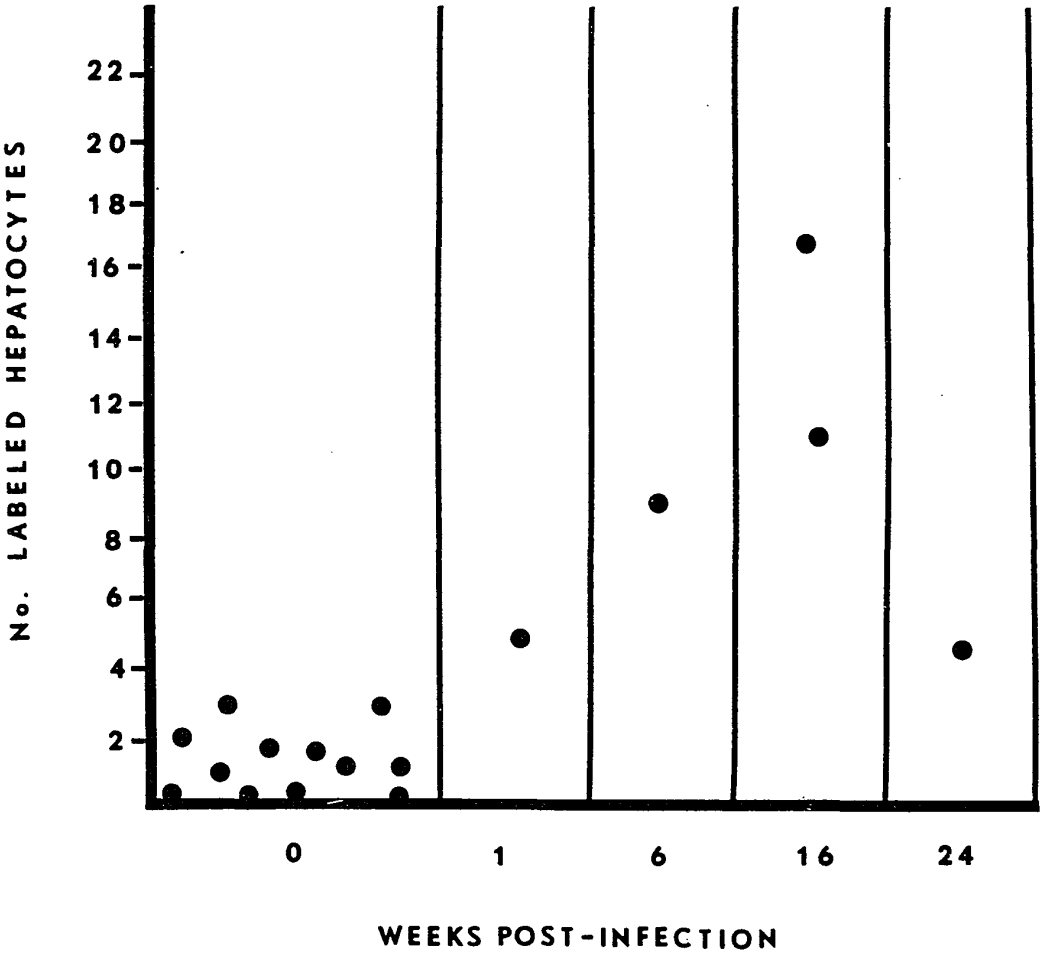


FIGURE 38

AUTORADIOGRAPHIC STUDY OF THE REGENERATION CAPACITY OF LIVER TISSUE DURING P. CONCINNUM INFECTION

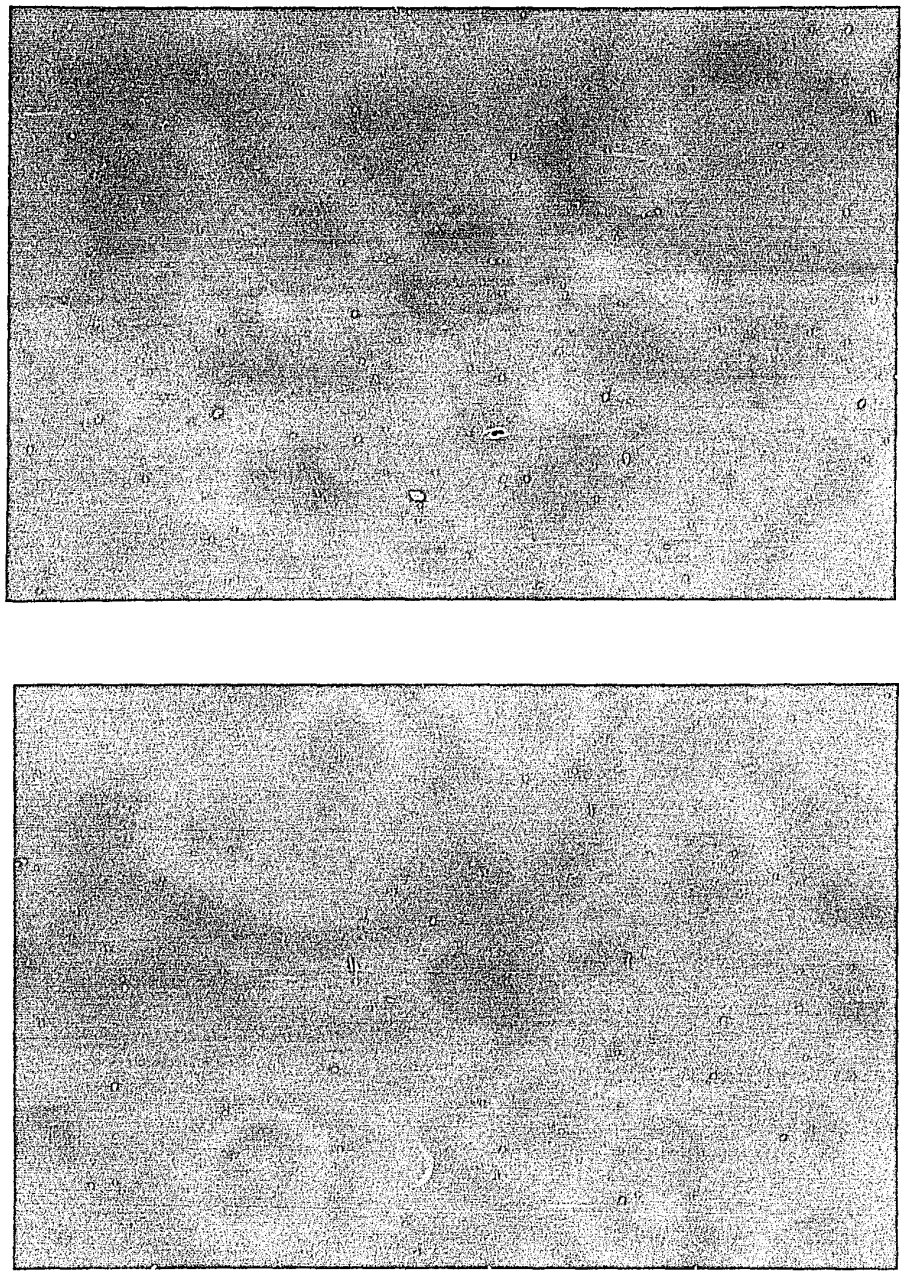


FIGURE 39

AN AUTORADIOGRAPH OF HEPATIC PARENCHYMAL CELLS
LABELED WITH TRITIATED THYMIDINE

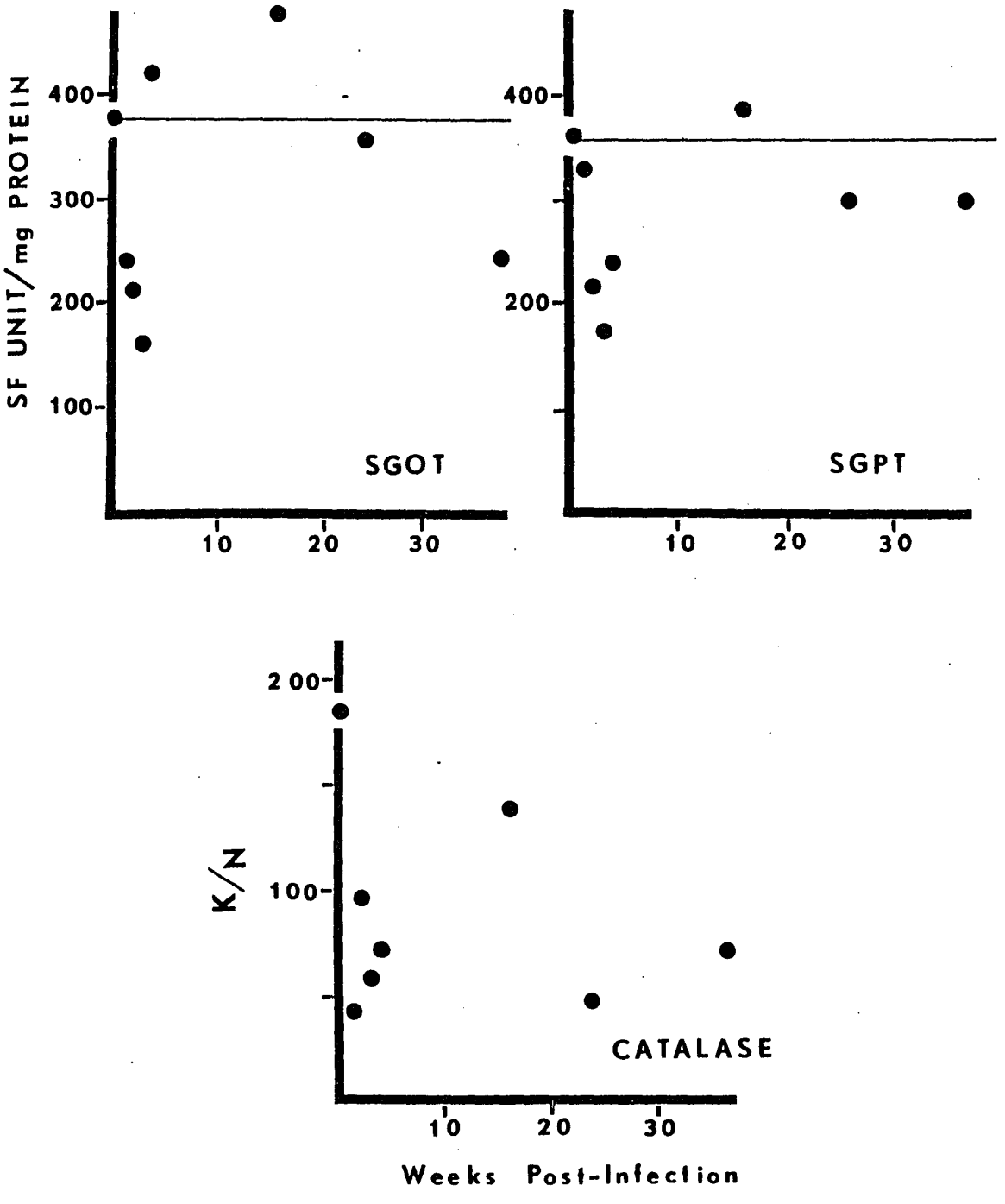


FIGURE 40

HEPATIC ENZYME LEVELS
DURING THE COURSE OF
P. CONCINNUM INFECTION

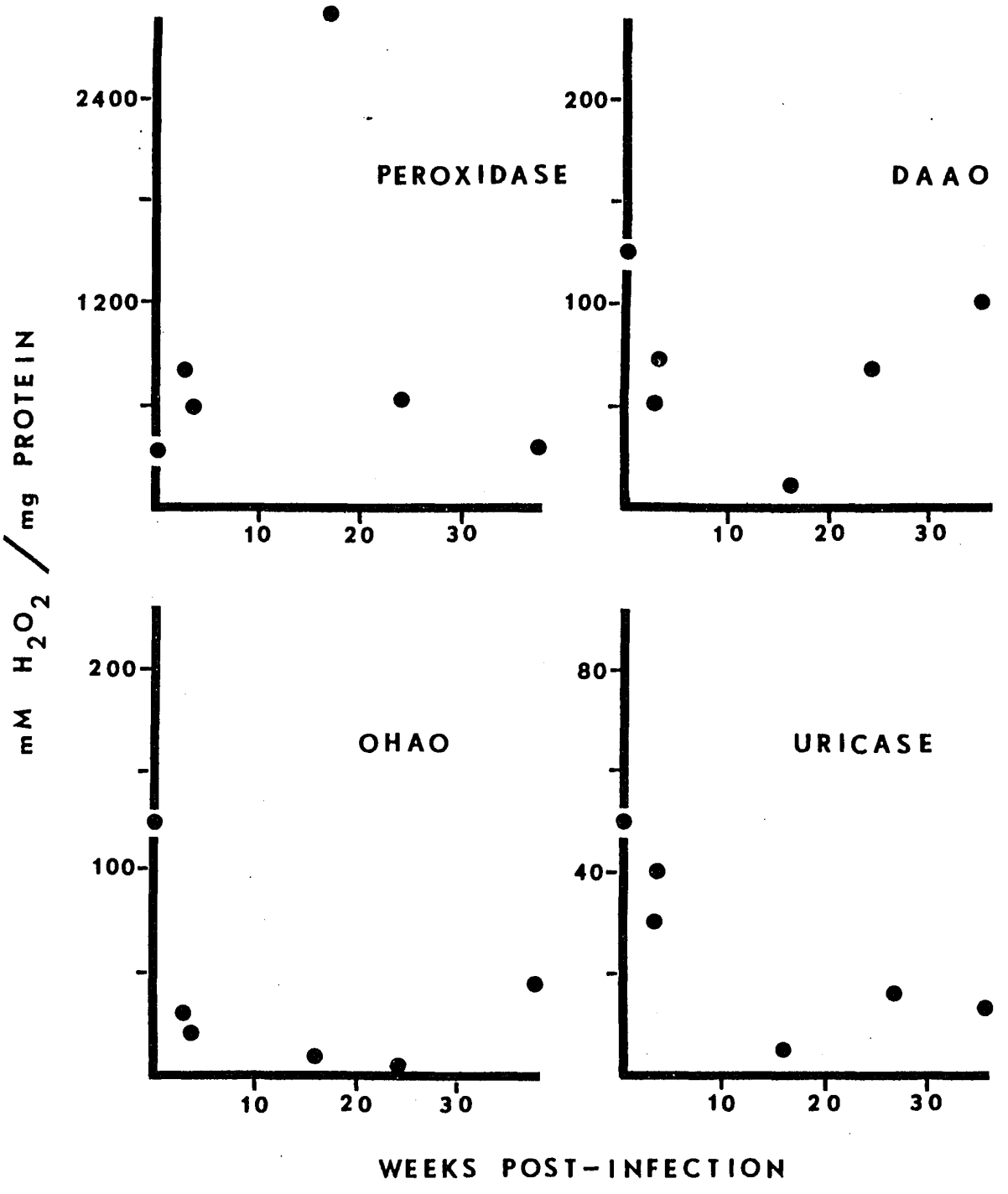


FIGURE 41

PEROXISOMAL ENZYME LEVELS IN HEPATIC TISSUE DURING THE COURSE OF P. CONCINNUM INFECTION.

returned to normal levels by 16 weeks post-infection. During the course of platynosomiasis, peroxidase enzyme levels were elevated compared to pre-infection values.

An approximately 30% higher level of catalase and OHAO enzyme activity has been reported in male rats when compared to females (Hruban et al., 1969). Uninfected cats however, presented no analogous sex dependent differences.

11. Hepatic and Serum Levels of Randomly Selected Cats

The enzymatic analysis of hepatic tissue in experimentally infected cats produced inconclusive data due to the small number of experimental animals and the wide range of values for these enzymes. Therefore, liver tissue, serum and feces were obtained at necropsy from 137 cats. Fecal samples helped identify the parasite burden of this population of cats. It was found that 53.3% of adult cats had Platynosomum concinnum infection, Table 5. The results suggest the disease is more prevalent in female cats (57.4%) than in males (47.8%); however, the average fluke burden was higher in males (202.0 + 432.7 flukes/cat) than females (171.5 + 330.2 flukes/cat). Kittens less than six months old were always negative.

The prevalence of four other groups of parasites was determined in this population of cats. The incidence of 1) hookworms--Ancyclostoma caninum; coccidia--Isospora felis, I. rivolta, I. begemina, Toxoplasma gondii; 3) lungworms--Aelurostrongylus abstrusus, Anafilaroides rostratus; and 4) roundworms--Toxocara canis, T. cati is also shown in Table 5. The distribution of parasite burdens in adult

TABLE 5

THE PREVALENCE OF SELECTED
FELINE PARASITES ON OAHU
1974

	ADULTS (TOTAL)	ADULT MALES	ADULT FEMALES	KITTENS
CAT LIVER FLUKE				
PLATYNOSOMUM CONCINNUM	53.3	47.8	57.4	0
AVE No FLUKES/LIVER ± SD	(191 ± 389)	(202 ± 433)	(172 ± 330)	0
HOOKWORM				
ANCYCLOSTOMA CANINUM	55.8	52.3	58.6	43.3
COCCIDIA				
ISOSPORA SPP	27.5	29.5	25.9	30.0
LUNGWORM				
AELUROSTRONGYLUS ANAFILAROIDES	14.7	11.4	17.2	0
ROUNDWORM				
TOXOCARA SPP	5.9	6.8	5.2	13.3

cats is shown in Figure 42. 82% of the cats surveyed had at least one parasitic infection.

Only 18.5% of the cats diagnosed as P. concinnum positive were free from infection by the other parasites studied. 43.1% of P. concinnum infected cats had one other parasite, 43.1% had two other parasites, 4.6% had three other parasites and 3.1% had four other parasites. There was no positive statistical correlation between P. concinnum infection and any other parasitic disease (Harris, 1975).

Serum transaminase enzyme activity (SGOT and SGPT) in cats with P. concinnum infection showed no correlation with fluke burden, Figure 43. Serum transaminase levels of cats free of platynosomiasis were also within normal ranges.

The liver tissue enzyme activity data is presented in Table 6. To insure that the presence of the other parasites did not influence the enzyme activities of hepatic tissue, regression analysis between each parasite or a combination of the parasites was made with each of the seven enzymes analyzed. Statistical analysis revealed no correlation between liver tissue enzyme values and the prevalence of other parasites studied. Also a correlation between the sex of the animal, its age (neonate, kitten or adult) or its physiological condition (pregnant, castrated, etc.) and the various enzyme activities was not found. Thus, the enzyme activities obtained could be averaged regardless of the physiological state of the animal, and the recorded enzyme values probably reflect the true effect of P. concinnum on liver metabolism.

Table 6 shows that only a few differences in enzyme activity

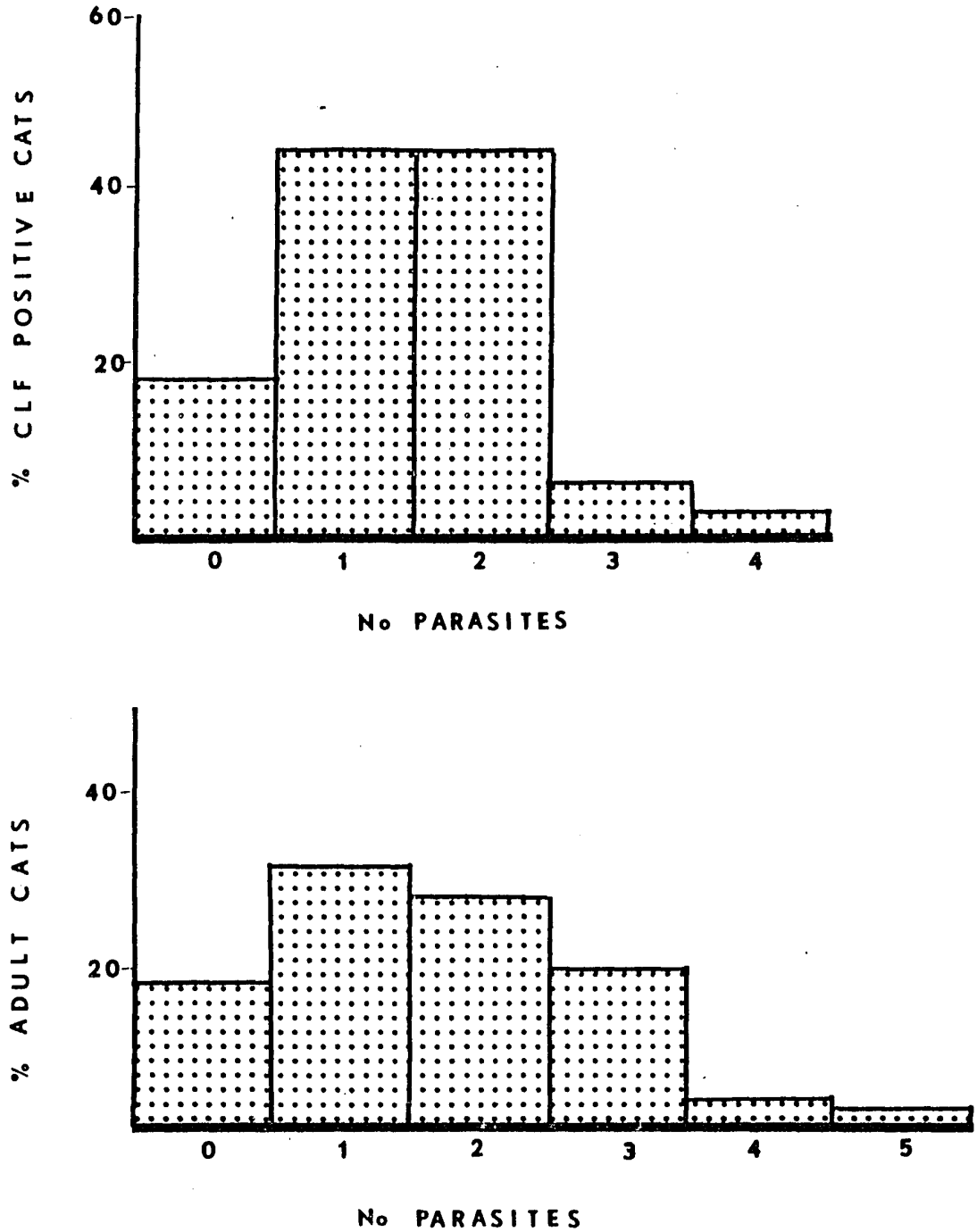


FIGURE 42

THE DISTRIBUTION OF PARASITE BURDEN IN CAT LIVER FLUKE POSITIVE ADULT CATS (UPPER GRAPH) AND IN A RANDOM SAMPLE OF ADULT CATS (LOWER GRAPH)

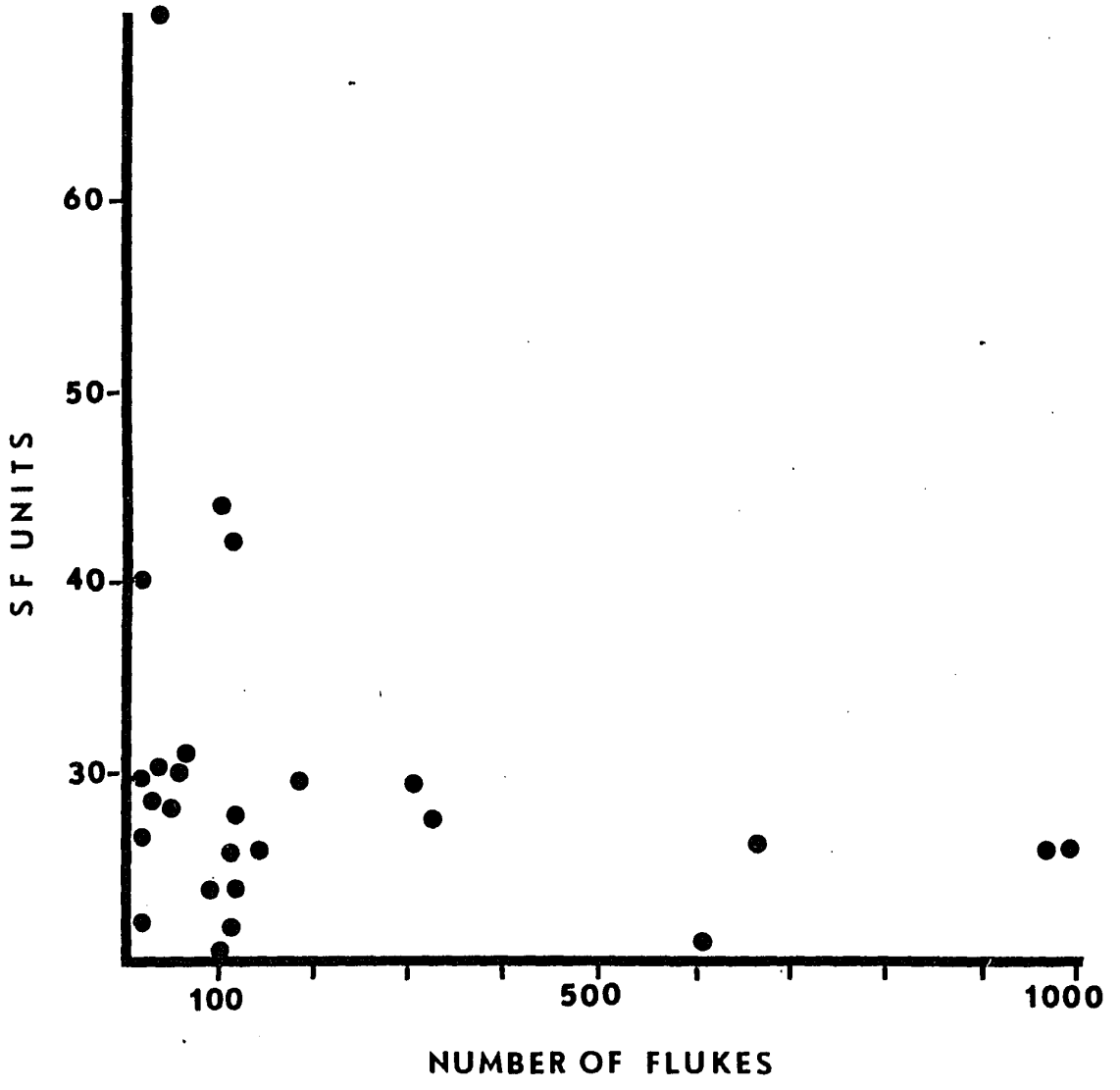


FIGURE 43

AVERAGE SGOT AND SGPT ACTIVITY LEVELS IN A RANDOM POPULATION OF CATS

TABLE 6

LEVEL OF HEPATIC ENZYMES ACTIVITY IN NORMAL AND PARASITIZED CATS

		No. Flukes/Liver				
		0	1-100	101-500	501 +	Value for all No. Cats
PT[a]	Males	424 ± 172	326 ± 187	344 ± 107	124 ± 101	317 ± 142
	Females	299 ± 180	297 ± 158	359 ± 235	649 ± 199	343 ± 193
OT[b]	Males	412 ± 188	361 ± 193	349 ± 100	950 ± 1138	405 ± 405
	Females	365 ± 241	337 ± 172	504 ± 454	868 ± 520	411 ± 347
PEROXIDASE[c]	Males	428 ± 266	312 ± 190	494 ± 272	139 ± 147	429 ± 219
	Females	355 ± 194	338 ± 208	598 ± 370	204 ± 118	373 ± 223
CATALASE[d]	Males	182 ± 57	161 ± 63	74 ± 91	234 ± 251	173 ± 116
	Females	178 ± 172	174 ± 79	175 ± 108	151 ± 140	174 ± 125
DAAO[e]	Males	116 ± 142	99 ± 77	129 ± 28	100 ± 71	116 ± 80
	Females	138 ± 101	118 ± 74	177 ± 230	175 ± 248	139 ± 163
OHAO[f]	Males	135 ± 119	127 ± 106	137 ± 78	40 ± 57	122 ± 90
	Females	126 ± 101	142 ± 179	97 ± 73	459 ± 535	155 ± 210
UO[g]	Males	52 ± 50	43 ± 36	46 ± 40	30 ± 28	42 ± 39
	Females	53 ± 36	97 ± 103	57 ± 27	140 ± 28	92 ± 49

a) Glutamic Pyruvate Transaminase (SF/mg Protein) * Mean ± Standard Deviation
b) Glutamic Oxalo Acetic Acid Transaminase (SF/mg Protein)
c) Peroxidase (mM H_2O_2/mg Protein
d) Catalase (K/N)
e) d-Amino Acid Oxidase (mM H_2O_2/mg Protein)
f) L-α-Hydroxy Acid Oxidase (mM H_2O_2/mg Protein)
g) Uricase (mM H_2O_2/mg Protein)

levels were apparent in P. concinnum infected cats when grouped according to fluke burden. There was an apparent depression of liver tissue GPT enzyme activity in male infected cats as compared to normal male cats, and an apparent increase of liver uricase activity in female infected cats as compared to normal female cats. Closer statistical scrutiny reveals however that these are not significant differences.

12. Results of Electrophoretic Analysis of Bile Proteins in Normal and Infected Cats

Cellulose acetate strip electrophoretic analysis of bile from normal cats as well as Platynosomum-infected cats revealed only two protein bands in most normal feline bile, but three bands were always present from infected cat bile. The third band (a slow gamma migrating protein) was detected in minute quantities in 20% of normal cats. Representative tracings from strip electrophoresis are shown in Figure 44.

When feline bile was analyzed using acrylamide disc electrophoresis as many as nine protein bands could be detected. In uninfected cats the number of bands and their characteristics were relatively uniform, whereas, the banding pattern of cats with platynosomiasis was more varied. Bands No's. 2, 3, 6 and 7 are more prominent in uninfected cats; while band 4 is absent (Figure 45). A larger amount of protein is generally present in bands 8—9 in cats with platynosomiasis. Figure 45 shows representative tracings from polyacrylamide gels.

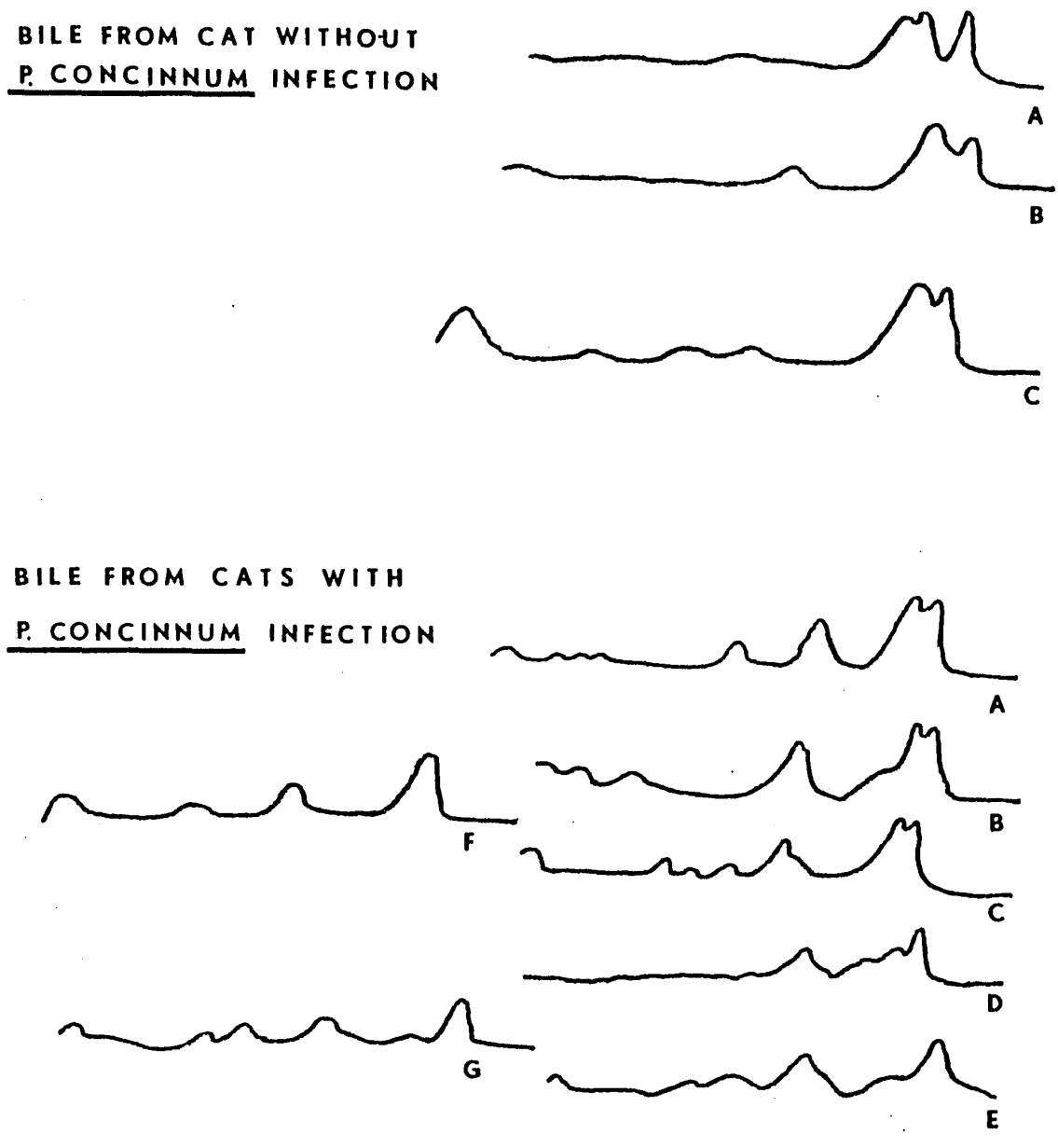


FIGURE 44

SCAN OF CELLULOSE ACETATE STRIP ELECTROPHORESIS
OF BILE FROM NORMAL AND CAT LIVER FLUKE POSITIVE CATS

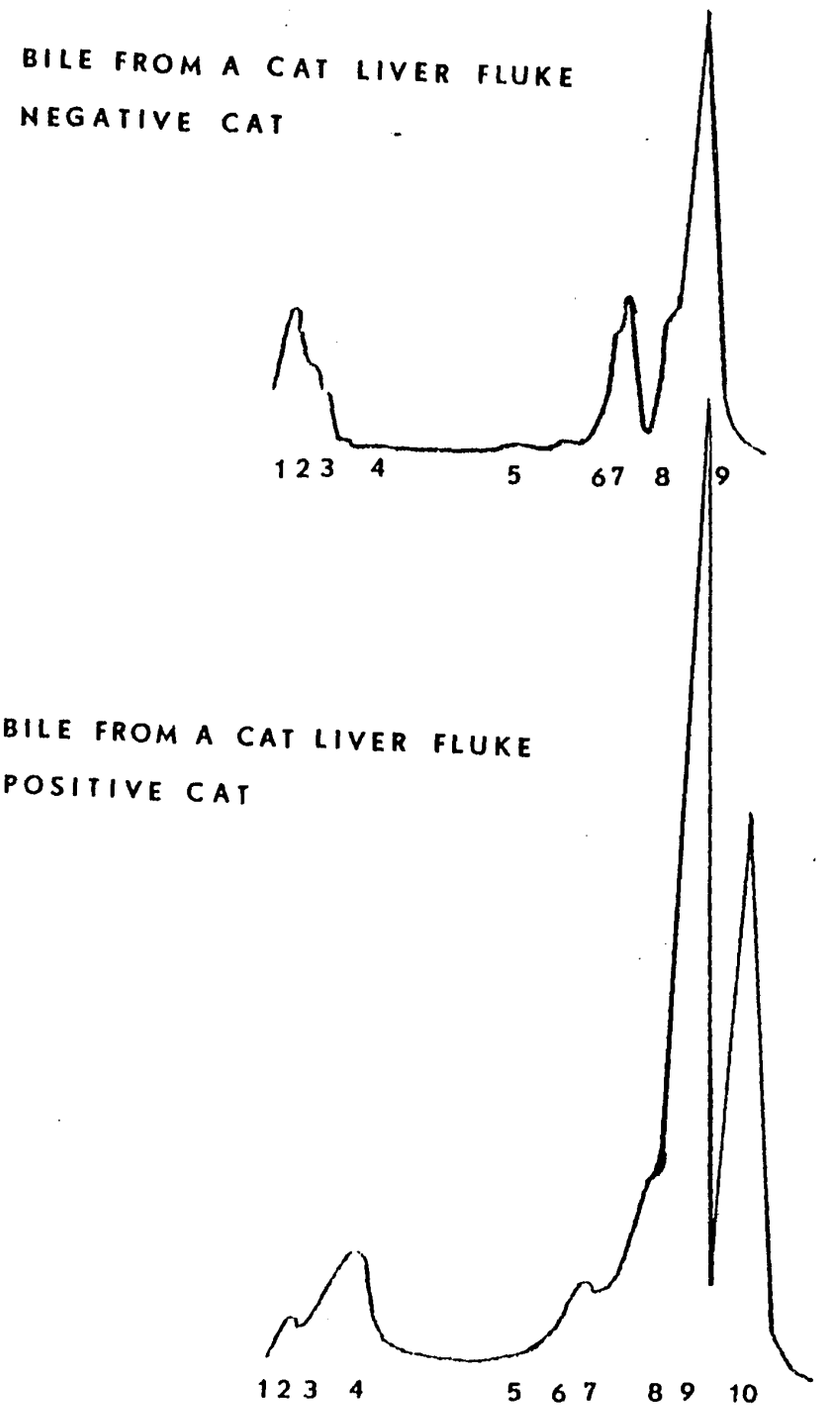


FIGURE 45

SCAN OF POLYACRYLAMIDE GEL ELECTROPHORESIS
OF BILE FROM NORMAL AND CAT LIVER FLUKE
POSITIVE CATS

An attempt was made to identify some of the additional bile proteins in infected cats. Bile from normal and P. concinnum infected cats was placed in double diffusion gel plates and run against rabbit-anti-adult P. concinnum antiserum, R-anti-P. concinnum-infective stage antiserum, serum from positively infected cats, serum from normal cats, and R-anti-cat IgG antiserum. Antiserum to cat albumins was not available. Double diffusion plates identified one of the additional proteins in P. concinnum infected cat bile as cat IgG. All other antisera failed to produce precipitin bands, thus, proteins of fluke origin or fluke metabolic products were not detected in bile from cats with platynosomiasis.

13. Antigenic Markers on the Surface of Platynosomum concinnum

A study of the antigenic material on the surface of P. concinnum was made. Double diffusion analysis showed rabbit antiserum prepared against the infective and adult stage of P. concinnum did not bind with extracts of adult flukes, serum from positively or negatively infected cats, feline erythrocytes, extracts of cat liver, or bile from cats with or without platynosomiasis. Thus, it appears P. concinnum does not share antigens in common with cats; it does not bind non-specific serum proteins or bile proteins to its surface, nor does it absorb feline glycoproteins which contain RBC antigens.

Since feline IgG was detected in bile of cats with platynosomiasis, a search for blocking immunoglobulins bound to the fluke surface was made. A radioimmunoabsorbance technic was used and the results are presented in Table 7. As can be seen, feline IgG is

TABLE 7

RADIOIMMUNOABSORBANCE ASSAY FOR THE PRESENCE OF FELINE IgG ON THE SURFACE OF *P. CONCINNUM*

TREATMENT	CPM/FLUKE	AVERAGE CPM/FLUKE
Normal Rabbit Serum + Sheep Anti-Rabbit IgG-^{125}I	1415 2209 1431 1713 1855 1650 1900 2147	1790 ± 296*
Rabbit Anti-Cat IgG + Sheep Anti-Rabbit IgG-^{125}I	3095 4381 3209 3516 3387	3517 ± 509
1:10 Diluted Normal Rabbit Serum + Sheep Anti-Rabbit IgG-^{125}I	1609 2197 2138 2261 1917 1454	1929 ± 332
1:10 Diluted Rabbit Anti-Cat IgG + Sheep Anti-Rabbit IgG-^{125}I	3229 2603 3096 3646 3646 2807 3491	3145 ± 397

*Average ± 1 standard deviation

undoubtedly bound to the surface of P. concinnum. The difference in counts per minute between the control and IgG treated flukes is highly significant (p=0.0001). The use of normal rabbit serum served as a control for non-specific electrostatic binding of rabbit serum proteins to the surface of the fluke.

14. Feline T and B Lymphocyte Subpopulations during Experimental P. concinnum Infection

During weeks 18--36 following P. concinnum experimental infection, T and B lymphocytes were assayed in Group A, B and C felines using both rosette formation and immunofluorescent staining technics. The distribution of feline T and B lymphocytes in these animals was not significantly altered during the period studied using either method. The data obtained using the rosette formation technic is presented in Table 8 while that obtained using the immunofluorescent staining technic is presented in Table 9.

15. Feline Lymphoblast Transformation during P. concinnum Infection

Viable lymphocytes were isolated from Group A, B and C cats 7 months after experimental P. concinnum infection and cultured in the presence of various concentrations of pokeweed mitogen (PWM), phytohemagglutinin (PHA), concanavalin A (ConA), and cat liver fluke extract (containing somatic and exoantigens). The data is presented in Figure 46. The effect of pokeweed mitogen (primarily a B cell stimulating mitogen in man) and phytohemagglutinin (primarily a T cell stimulating mitogen in man) on viable lymphocytes obtained from both control and P. concinnum parasitized cats is presented in Table 10.

TABLE 8

THE NUMBER OF T AND B LYMPHOCYTES
AS DETERMINED BY ROSETTE FORMATION IN
CONTROL AND PARASITIZED CATS

CAT #		EAC ROSETTE %	E ROSETTE %
GROUP A	1	42.8	34.0
	2	41.0	17.0
	5	39.5	24.1
	6	38.5	39.0
	7	26.3	34.0
	8	43.4	34.0
	AVE.	38.6 ± 6.3	29.6 ± 8.9
GROUP B	13	48.0	24.0
	14	58.3	29.5
	15	49.5	13.3
	16	44.2	43.0
	18	37.5	26.9
	19	50.3	47.5
	20	44.6	12.3
	21	36.5	25.0
	AVE.	46.1 ± 7.1	27.7 ± 12.5
GROUP C	22	33.0	32.5
	23	38.8	40.0
	24	49.1	30.0
	25	32.0	34.0
	27	29.5	40.0
	28	44.8	38.0
	29	45.0	26.3
	AVE.	38.9 ± 7.6	34.4 ± 5.2

TABLE 9

THE NUMBER OF T AND B LYMPHOCYTES IN CONTROL AND PARASIZED CATS AS DETERMINED BY IMMUNOFLUORESCENT STAINING

Group	Cat. No.	B Cells Anti-IgG	T-Cells Anti-Thymus
A	5	41.2	48.0
	6	41.0	43.0
	Average	41.1	45.5
B	14	43.5	37
	18	52.0	42.0
	19	49.0	46.0
	Average	48.2	41.7
C	22	43.5	40.5
	25	45.5	49.0
	29	50.0	24.0
	Average	46.3	37.8

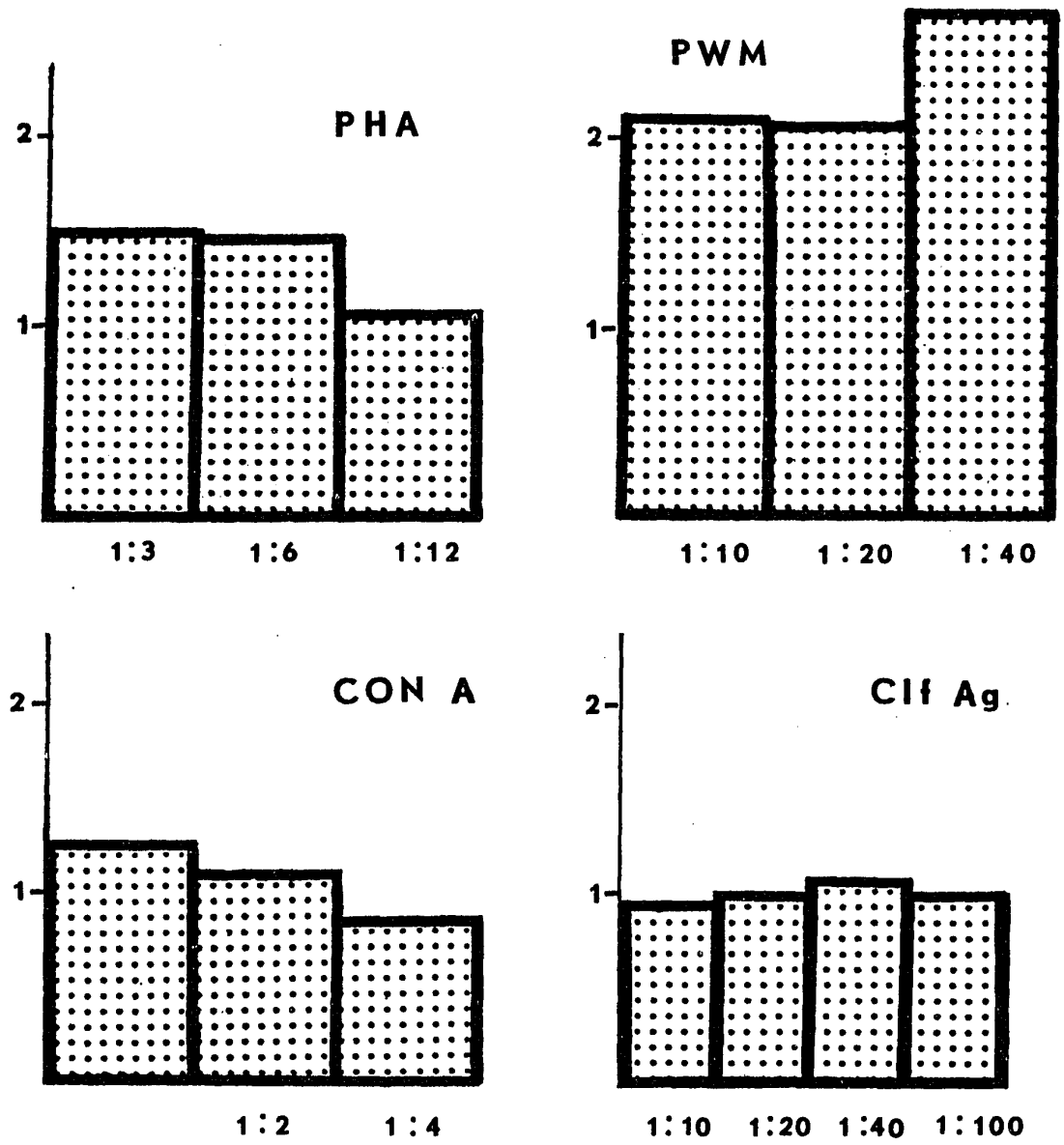


FIGURE 46

RESPONSE OF FELINE LYMPHOCYTES TO VARIOUS CONCENTRATIONS OF MITOGENS

TABLE 10

THE EFFECT OF MITOGENS ON FELINE
LYMPHOCYTE BLAST TRANSFORMATION

Group	Cat No.	PWM	PHA	CON. A	*P. concinnum* Extract			
					0.1m	0.05ml	0.025ml	0.01ml
A	1	1.5	1.14	1.0	-	-	-	0.88
	2	2.5	1.5	-	-	-	-	-
	5	1.1	1.3	-	-	-	-	-
	7	3.5	2.1	1.1	1.1	-	-	1.1
	8	1.2	0.9	-	1.1	-	-	1.1
	Ave.	2.0±1.0*	1.4±0.5	1.1	1.1	-	-	1.0±.1
B	13	2.4	2.5	1.1	1.1	-	-	-
	16	1.2	1.2	1.0	-	-	-	-
	15	1.1	1.0	1.0	-	-	-	-
	19	1.0	1.0	-	-	-	-	-
	Ave.	1.4±0.7	1.4±0.7	1.0±0.1	1.1	-	-	-
C	23	1.9	1.1	1.7	-	-	-	-
	24	1.2	1.0	1.3	0.86	0.88	1.1	-
	25	1.2	1.2	-	-	-	-	-
	28	2.0	1.2	0.9	1.1	-	-	-
	Ave.	1.6±0.4	1.1±0.1	1.3±0.4	1.0	0.88	1.1	-

* Average ± 1 standard deviation.

Concanavalin A did not induce blast transformation in feline lymphocytes. In addition, isolated viable lymphocytes of both control as well as P. concinnum infected cats did not respond to various concentrations of cat liver fluke extract used as a possible mitogenic or antigenic substance.

16. Secondary Challenge of Naturally Infected Cats (Group E) with P. concinnum Infective Stages

Three naturally infected cats received an orally administered secondary challenge dose of P. concinnum infective stages. Three weeks post-infection cat #32 was euthanised and necropsied. Post-mortem examination revealed all organs were of normal pathology except for dilatation of hepatic bile ducts as a result of the initial infection. Twenty-one adult flukes from the primary infection and 245 juvenile flukes from the secondary infection (51.4% of the infective stages experimentally administered) were recovered. Eight weeks post-infection cats No's. 33 and 34 were sacrificed. No abnormal pathology was found at necropsy except for bile duct enlargement characteristic of chronic platynosomiasis. Four hundred seventeen adult flukes from the primary infection and 119 maturing flukes from the secondary experimental infection were present in the liver of cat No. 33. Thus, only 23.0% of the experimentally administered infective dose was recovered. Careful examination of the liver from cat No. 34 revealed the presence of 2 adult flukes (primary) and 552 maturing juvenile flukes (secondary). 77.5% of the initial experimental dose was recovered 8 weeks post-infection from this cat.

17. Cell Mediated Immunity in Platynosomum concinnum Infection in Juvenile Cats

Cell mediated immunity during P. concinnum infection was studied using six littermate juvenile cats, 13 months old, the adult queen cat served as a source of viable lymphocytes and serum for inoculation into these juvenile cats. The results of in vivo studies of cell mediated immunity are presented below. Two juvenile cats A and B served as controls and were inoculated only with 20 ml saline prior to infection, juvenile cats C and D received 20 ml I.P. serum from an adult cat liver fluke positive donor, and cats E and F were inoculated (intraperitoneally) with viable sensitized lymphocytes.

A. Clinical Signs and Symptoms

All six experimental animals (A, B, C, D, E and F) remained in good health; no clinical signs or symptoms attributable to platynosomiasis were noted. The weight changes for each of the six animals during the course of infection are given in Appendix A; weight loss was not noted.

B. Hematologic, Serologic, and Serum Biochemical Findings

The hematologic data is presented in Figures 47 and 48. No elevation in serum transaminase enzyme activity levels (SGOT and SGPT) nor in total leucocyte count during the 8 weeks of infection was noted in cats A and B (saline controls). However, an eosinophilia, most apparent 1 month after infection was noted. Cats C and D (serum recipients) presented only a slight eosinophilia but elevated serum transaminase enzyme activity levels beginning 32 days post infection. Cats E and F (viable lymphocyte recipients) presented persistent

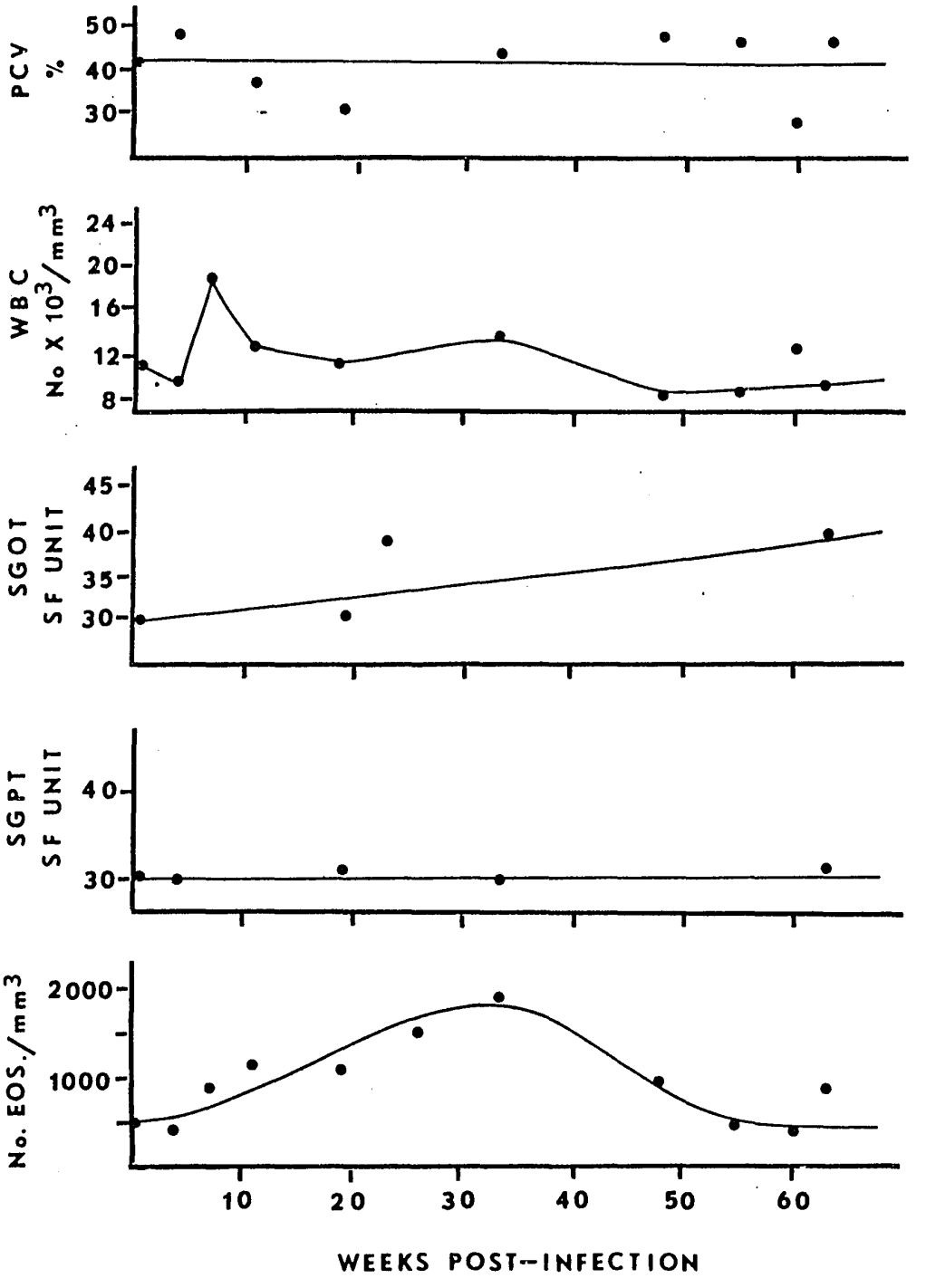


FIGURE 47

HEMATOLOGIC VALUES FOR CATS A AND B
(NO PRETREATMENT) DURING P. CONCINNUM INFECTION

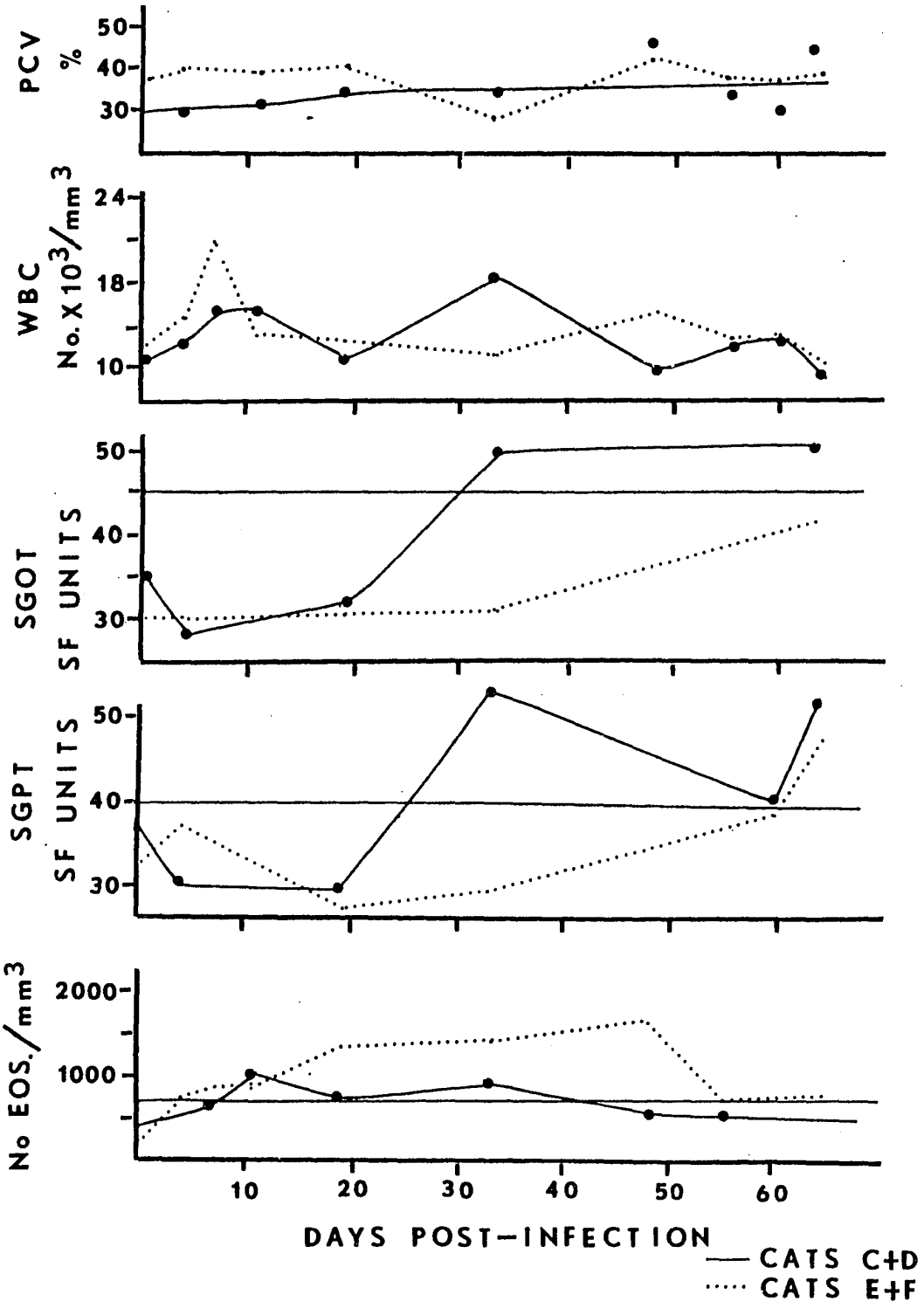


FIGURE 48

HEMATOLOGIC VALUES FOR CATS C AND D (SERUM RECIPIENTS) AND E AND F (LYMPHOCYTE RECIPIENTS) DURING THE COURSE OF INFECTION

eosinophilia lasting 20-50 days post-infection. The serum transaminase enzyme activity levels in these cats increased slightly during the period studied but in general stayed within the normal range. A brief leucocytosis occurred one week post-infection.

C. Necropsy Results

Two months after experimental P. concinnum infection the cats were euthanised and necropsied.

Cat A: The liver was slightly enlarged. Very few bile ducts were dilated. No changes were noted in other organs.

Cat B: The liver appeared normal. Only a few bile ducts in the median and right lobes of the liver were enlarged. Other organs were normal.

Cat C: Enlarged bile ducts were present in all lobes of the liver and biliary stasis due to dark bile was noted. This feline presented congenital unilateral nephrosclerosis with contraction and scarring of the right kidney. Other organs were normal.

Cat D: The liver was normal except for enlarged bile ducts throughout all lobes. Other organs were normal.

Cat E: The liver and bile ducts were normal. Dark bile was present in the gall bladder which was not noticeably distended. Other organs were normal.

Cat F: No gross pathologic changes were noted in any organ.

D. The Number of Flukes Recovered at Necropsy and their Distribution within the Biliary Tract

The number of P. concinnum flukes recovered is shown in Table 11. The distribution of these flukes within the liver is

TABLE 11

FLUKE BURDEN OF CONTROL, SERUM, AND LYMPHOCYTE RECIPIENT CATS

CAT TREATMENT	CAT #	PERCENT FLUKES RECOVERED	AVERAGE NUMBER FLUKES RECOVERED	PERCENT PROTECTION
CONTROLS	A	31	22	-
	B	13		
SERUM	C	33	23.5	-6.8
	D	14		
LYMPHOCYTES	E	13	9.5	56.8
	F	6		

indicated in Table 12. An average of 22% of the infective stages were recovered from saline control cats, 23.5% from serum recipients and 9.5% from cats receiving viable lymphocytes from the P. concinnum sensitized donor. The flukes were distributed within the liver lobes equally in all cats. However, the number of flukes found in the gall bladder differed in these variously treated groups. No flukes were present in the gall bladder of saline control of serum recipient cats, but 16.1% of the flukes recovered were found in the gall bladder of lymphocyte recipient cats.

E. Developmental Stage and Size of Platynosomum concinnum

The majority of the flukes recovered were mature and had the same morphology as those in Figure 37. Flukes in the four hepatic lobes varied in size (Table 13). The flukes in the left hepatic lobes of control, serum, and lymphocyte recipient cats were the same size; however, those in the right lobe were markedly smaller in the lymphocyte treated cats. The flukes from cats E and F were in general smaller than those found in the other cats.

TABLE 13

AVERAGE LENGTH AND WIDTH OF FLUKES RECOVERED FROM IN VIVO STUDY

	Median Lobe	Right Lobe	Caudate Lobe	Left Lobe	Gall Bladder
Controls	2.8 x 0.9	2.6 x 0.94	2.1 x 0.8	2.3 x 0.85	-
Serum	2.2 x 0.99	3.0 x 0.91	3.1 x 0.97	2.2 x 0.87	-
Lymphocyte	2.0 x 0.75	1.4 x 0.6	3.0 x 0.8	2.3 x 0.85	1.9 x 0.7

TABLE 12

THE DISTRIBUTION OF FLUKES IN THE LIVER OF CONTROL, SERUM, AND LYMPHOCYTE RECIPIENT CATS

	% FLUKES/LOBE					No. FLUKES/GRAM HEPATIC TISSUE			
	Median	Right	Caudate	Left	Gall Bladder	Median Lobe	Right Lobe	Caudate Lobe	Left Lobe
CONTROLS									
51	38.5	15.4	7.7	38.5	0	0.52	0.13	0.15	0.22
53	38.7	29.0	3.2	29.0	0	0.69	0.45	0.22	0.35
Average	38.6	22.2	5.5	33.8	0	0.61	0.29	0.19	0.29
SERUM									
47	57.6	15.2	9.0	18.2	0	1.65	0.38	0.60	0.34
54	7.1	35.7	14.3	42.9	0	0.08	0.45	0.33	0.29
Average	32.4*	25.5	11.7	30.5	0	0.87	0.42	0.47	0.32
CELLS									
45	33.3	16.7	0	33.3	16.7	0.13	0.06	0	0.07
	0.0	31.0	30.0	23.1	15.4	0.0	0.28	0.57	0.11
Average	16.7*	23.9	15.0	28.2	16.1	0.07	0.17	0.29	0.09

29.5% of the flukes from lymphocyte recipient animals were immature compared to 15.6% from serum recipients and only 2.6% from saline control cats (Table 14).

TABLE 14

AVERAGE SURFACE AREA AND PERCENTAGE OF IMMATURE FLUKES RECOVERED FROM UNTREATED, SERUM AND LYMPHOCYTE RECIPIENTS

	Average Surface Area/Fluke (mm^2)	Percent Immature Flukes Recovered
Controls	2.1 mm^2	2.6
Serum	2.4 mm^2	15.6
Lymphocyte	1.6 mm^2	29.5

F. Rosette Formation of Peripheral Blood Lymphocytes

E rosette studies to assay the number of T cells present were made prior to and 50 days following infection. The data is presented in Table 15. The number of T lymphocytes found in the lymphocyte recipient cats was the greatest encountered to date by the author.

TABLE 15

ROSETTE FORMATION OF PERIPHERAL BLOOD
LYMPHOCYTES 50 DAYS POST-EXPERIMENTAL INFECTION

	ROSETTE FORMATION			
	Baseline		50 Days Post-Infection	
Cat. No.	EAC	E	EAC	E
A	54*	26	-	45.5
B	44	20	-	-
C	44	23	-	-
D	44	40	-	28.5
E	37	23	-	-
F	32	16	-	56.5

*Values expressed as percentages

DISCUSSION AND CONCLUSION

Current studies on the host-parasite relationship between the liver fluke, Platynosomum concinnum and the domestic cat, Felis catus have lead to some interesting observations and conclusions concerning this host-parasite association. By definition, an efficient parasite does not cause the death or endanger the survival of its host. In this respect, P. concinnum is a successful digenean parasite.

Dicrocoeliidae are among some of the least studied Trematodes, in part due to the complexity of their life cycles and in part, due to thir relatively minor economic importance. Several unique biological features of P. concinnum have been observed in this study. One of these features concerns the infective stage found in toads and lizards. The stage found in the toad has been referred to as an infective stage in this study. Maldonado (1945) used this term throughout his report for the stage found in the biliary tract of the Anolis lizard. However, in his illustrations he labeled this stage a metacercaria. Parasitologists consider a metacercaria to be an infective stage with arrested development. Morphologically, the infective stage in the toad fits the general description presented for Trematode metacercaria (Erasmus, 1972). However, all metacercaria except two members of the Order Strigeatoidea (subfamily Diplostomatidae and suborder Brabchylaimata), encyst either within another intermediate host or in the environment. In Dicrocoeliidae the morphology of the encysted metacercaria and that of the juvenile fluke which emerges from the cyst are identical. It is possible an encysted form of P. concinnum occurs in nature and that this juvenile

fluke infects a third intermediate host. Since any single stage in a Trematode life cycle is often defined in terms of the stages which precede and succeed it, the positive identity of the parasitic stage found in the amphibian and reptilian intermediate hosts cannot be stated with certainty. It is however believed that the toad, Bufo marinus, is a parentenic host and not an essential member of the life cycle of P. concinnum. It is unlikely that in nature cats could become infected by eating toad liver from toads whose parotid gland toxin is lethal to many species (Otani, 1969).

The experimental studies simulated as closely as possible the natural course of P. concinnum infection by the oral administration of 100-2000 infective stages to various groups of cats. The average parasite burden in infected pets and stray cats was estimated to be 202 flukes with a range from 1 to 2000. Thus the experimentally administered low dose of 200 infective stages was near the lower end of the range found in nature, while at the other end of the scale the high dose cats were administered the experimental dose of about 2000 infective stages. There were, of course, certain parameters which were not mimicked in the experimentally induced infections. Rather than the brief period required for experimental exposure, cats in nature probably acquire their total fluke burden over a prolonged period since an infected skink or gecko has about ten infective stages within its liver. Furthermore many cats in nature lead a semi-feral life with inadequate shelter and food, and most have other parasitic infections. Animals in this study however were housed in constant temperature environments, free of external stress, fed a high protein diet, and were free from

other parasitic infections. Thus we might expect P. concinnum infection to be more severe in nature because of these synergistic factors. Most cats observed during this study with naturally acquired platynosomiasis, however, do not appear clinically ill.

Mild clinical signs were noted in only 5 of the 26 experimentally infected animals. These signs were revealed by careful daily notation of changes in the behavioral patterns of each cat. Most probably the average pet owner would not have noted some of these outward signs of illness in his pet. Transient and mild signs of inappetence and lethargy occurred before diagnosis of platynosomiasis by detection of ova in the feces. Thus these clinical signs corresponded to the period when flukes were becoming established and maturing in the biliary tract of the host. Jaundice, weight loss, emaciation, vomiting, diarrhea, and death as reported in the literature were not noted in this experimental study. Observation of these signs by others could be attributed to other concomitant pathologic conditions.

Immature ova of P. concinnum were detected in the feces as early as eight weeks post-infection in some cats and all cats fed infected toad livers passed ova in the fecal samples by week 12. Thus, there was a prepatent period without the passage of ova of 10 to 12 weeks. Mature P. concinnum ova were present in fecal samples of some animals as early as week 10. This correlates well with the developmental stages of the parasites. Immature ova found in the fecal samples of the infected cats were very important in establishing the very early diagnosis of the disease. Immature ova released into the feces by the fluke have not been found to undergo further development and thus

apparently play an unimportant role in the life cycle.

After 5-6 weeks Toxocara cati ova were present in the feces of all cats fed toad livers as the source of infective P. concinnum stages. The second larval stage of T. cati has been reported in earthworms, cockroaches, birds and mice (Sprent, 1956). When a definitive host ingests the second stage larvae, the parasite remains in the intestinal tract and does not migrate within the host as happens when ova are ingested. T. cati ova can then be detected in the feces as early as 3-6 weeks post-infection when second stage larvae are ingested. Thus, the toad B. marinus was also shown here to be a new intermediate host for T. cati. The experimental cats were administered piperazine orally, but careful examination of the feces post-treatment revealed that only 1-8 adult T. cati roundworms were passed by each cat. It is significant that approximately three times as many roundworms were recovered from the feces of the cats that had received high dose as compared to low dose infective stages from toad liver. Thus, the evidence that the toad B. marinus is a new intermediate host in the life cycle of T. cati rests on firm ground.

There was no difference in the hematologic values, gross pathology, or fluke burden noted in cats treated with piperazine and those which received no treatment. Furthermore 2 cats were experimentally infected with isolated metacercariae from toad livers which were administered orally via stomach tube. In these cats, the hematologic values were shown to be statistically the same as those which had also developed T. cati infection. The data support the conclusion that T. cati infection had no effect on the course of platynosmiasis.

Pathologic changes of the liver caused by the establishment and maturation of P. concinnum within the biliary tract resulted in alterations in the hematologic and serum biochemical profiles of infected cats. Within three weeks following experimental infection the flukes had doubled in size. As a result, major bile ducts became dilated and adenomatous hyperplasia was initiated. The enlarged bile ducts contributed to parenchymal cell damage and was reflected in elevated serum transaminase enzyme activity levels.

Cellular infiltration was noted in response to the biliary damage. Mechanical damage to the bile duct lining where the fluke attach would have been sufficient to release histamine, the C_3 component of complement, bradykinin, or other substances, which in turn would provide the chemotactic factors necessary for infiltration by polymorphonuclear leucocytes, lymphocytes and macrophages. The eosinophilia present around the bile ducts probably was a response to a metabolic product produced by the parasite which served as an exoantigen. As the fluke matured and invaded even smaller bile ducts, intermittent pre- and post-ductal biliary stasis occurred. Bile ducts engorged under the localized pressure and chemical irritation by bile lead to continued leakage of hepatic transaminase enzymes into the blood.

During the first six weeks of infection, the gall bladder became distended with bile. The mechanism whereby the gall bladder becomes a thin walled, tense structure is not completely clear. In clonorchis infections flukes are thought to plug the common bile duct causing total obstruction of the biliary tract (Hou, 1955). This probably does not occur in P. concinnum infection due to the low number of flukes

residing in the common bile duct and their smaller size. One month after infection hepatic and mesenteric lymph nodes were, in general, enlarged. However, in only one cat (No. 26) were the hepatic lymph nodes enlarged sufficiently to cause partial mechanical obstruction of the common bile duct resulting in gall bladder dilatation. A neurogenic pain response mechanism may be involved in producing gall bladder enlargement in platynosomiasis. Relaxation of the bile duct sphincter and the release of bile is regulated by hepatic secretion, gall bladder tone and major bile duct pressure. Local irritation of the common bile duct may produce dyskinesia resulting in the retention of bile. Migration and maturation of the fluke would be sufficient to elicit a neurogenic response creating a spasm of the biliary sphincter and duodenal musculature. Thus, complete blockage of the common bile duct would result for extended periods of time. The obstructed bile flow could result in an aneurism of the cystic duct sufficient to mimic the presence of a secondary gall bladder and the wall of the gall bladder itself would then appear thin and stretched. The increased volume of bile in the gall bladder is in turn reflected in the biliary stasis noted histologically. If the bile duct blockade is severe and prolonged enough, an elevation of serum total bilirubin levels would be recorded. This pathologic state was noted in only four of the experimentally infected cats. An increase in serum icterus index also monitored these changes.

Serum alkaline phosphatase enzyme activity levels are not elevated in cats at this time as might be expected. Apparently the feline hepatic tissue alkaline phosphatase enzyme is not liberated during

partial biliary obstruction as it is in other species (Sherlock, 1973, Leevy, 1963).

Three months after infection the flukes have reached maturity and their growth rate has been greatly reduced. Enlargement of the bile ducts and damage to liver parenchyma has been essentially completed. Bile ducts throughout the liver lobes have already become enlarged. Fibrotic connective tissue has started to form a "wall" around the bile ducts thus reducing the flow of bile and fluke metabolic products into the parenchymal tissue. The formation of fibrous connective tissue resulted in two changes: 1) cellular infiltration was reduced around the bile ducts and the lymph nodes decreased in size since fewer metabolic products or exoantigens were released into the area, and 2) the mechanical support around the bile ducts coupled with decrease in biliary tension resulted in release of pressure of the bile ducts on hepatic parenchymal cells. The leakage of liver parenchymal transaminase enzymes into the blood was lessened and by four months post-infection the serum transaminase enzyme activity had returned to normal levels.

During the remaining six months of the study, adenomatous hyperplasia continued slowly, cellular infiltrates were present around the secondary bile ducts and wide zones of connective tissue continued to form, separating the inflamed bile ducts from the parenchyma.

The course of liver pathology noted was similar to that seen in fascioliasis, but in that disease, calcification of the bile ducts also occurs, walling off the parasite. Dawes (1964) feels that this process of calcification is an active host mechanism whereby the fluke

is segregated within a calcareous wall. Calcification does not seem necessary in feline P. concinnum infection to accomplish the same end.

The pathology seen in platynosomiasis appears to be simple and direct. However, other complicating factors seen in clonorchiasis were not observed. The bile remained clear and histologic material gave no indication of bacterial infection involvement. Microabscesses around ova were not observed in paranchymal tissue nor were "bile stones" noted in the bile. It would appear the pathology of C. sinensis in man and P. concinnum in cats are basically identical; however, reports on clonorchis pathology are confusing and inconsistent (Hou, 1955, Viranuvatti et al., 1971) especially because of the aforementioned complicating factors.

The major alterations in the hematologic profiles of cats experimentally infected with P. concinnum were a persistent eosinophilia, a sharp elevation of bilirubin and serum glutamic oxaloacetic acid and glutamic pyruvic acid transaminase enzyme activity levels. A slight leucocytosis occurred in several cats during the first three months post-infection but this was not a general finding.

Cats experimentally infected did not become anemic, nor did the serum albumin or total protein levels decrease during infection. Histologically the epithelium and stroma around the bile ducts remained intact. There was no direct evidence for leakage of whole blood or serum protein into the bile. Feline red blood cells were not present in the caeca of any of the flukes examined microscopically.

All kidneys examined both at necropsy and histologically were normal except for those of cat No. 26. The blood urea nitrogen values

fluctuated slightly during the course of infection and appeared to be slightly lower during the early phase of the disease. The extent of kidney damage stemming from platynosmiasis infection is probably minor and of secondary importance.

Flukes were not recovered from ectopic sites in experimentally infected cats. Flukes were recovered from the common bile duct but not from the pancreatic duct or the pancreas itself. No experimentally infected cat developed diabetis mellitis.

In summary the pathology produced by P. concinnum infection was limited to the liver and mesenteric lymph nodes. Necropsy results and hematologic and serum biochemical evaluations suggest other tissues were not involved.

The question of parenchymal involvement is an important one. Liver parenchymal necrosis with scarring is present in fascioliasis (Dawes et al., 1964) and liver portal-hypertension is present in Schistosomaisis (Warren, 1972). In P. concinnum infections the parenchyma appears normal histologically; however, the regenerative capacity or the metabolic pathways could have been altered.

Higgins et al. (1932) in studies using rats and Mann et al. (1931) using dogs concluded that little or no parenchymal liver regeneration occurred when biliary flow was obstructed due to bile duct ligation. Furthermore, Ferguson et al. (1949) showed that protein synthesis was not effected by partial ligation of the common bile duct nor were the number of parenchymal cells undergoing mitosis altered. The effect of P. concinnum infection on liver involvement was examined using radioautography technics. Theoretically the number of DNA synthesizing cells

is not equally distributed throughout the liver. If a bile duct becomes obstructed, another may proliferate, or the parenchyma around the blocked bile duct may atrophy. Thus, a biopsy sample from a newly proliferating duct area would show higher DNA synthesizing activity than a sample taken from an area with atrophy. For this reason liver tissue was analyzed from an area equivalent to the biopsy site as well as from the center of the left hepatic lobe of all cats at necropsy in order to obtain comparable results. The number of labeled cells in hepatic tissue of parasitized cats did not vary significantly from those in biopsied material. The liver therefore maintained its ability to synthesize DNA throughout the course of infection. Cat No. 26 showed signs of partial biliary obstruction at the time of necropsy. The number of cells capable of synthesizing DNA was approximately the same as those of the biopsy samples. The failure to find labeled ductal cells is an enigma. Perhaps feline bile duct epithelial cells require more than 90 minutes to incorporate thymidine. This period, however, is sufficient for the labelling of human bile duct cells (Leevy, 1963). Autoradiography studies suggest biliary stasis during platynosomiasis was insufficient to suppress the liver regeneration capacity of feline liver; however, P. concinnum infection triggered the release of chemical factors into the blood resulting in an increased regeneration of liver parenchymal cells (MacDonald, 1960).

Liver parenchymal enzyme activity showed minor changes during the course of infection. During the early stage of infection, while the flukes were growing and maturing in the bile ducts, a slight decrease in the hepatic transaminase levels was noted with a parallel increase

in serum transaminase levels.

The localization of liver parenchymal cell peroxisomal enzyme system has been studied in cats by several investigators (Truszkowski et al., 1933, Birkhofer et al., 1940, Holtz et al., 1942). Studies using the electron microscope showed a coarsely polytubular core within feline peroxisomes indicating the presence of uricase (Hruban et al., 1969). Catalase and d-amino acid oxidase have also been reported but the concentration of DAAO had not previously been determined. The data presented in this dissertation is the first report concerning the presence of 1-α-hydroxy acid oxidase in feline peroxisomes.

Cats experimentally infected with P. concinnum showed a general decrease in hepatic peroxisomal enzyme activity. This was rather unexpected. It appears that neither DAAO, OHAO, or uricase are responsible for producing a large amount of hydrogen peroxide during the course of infection. However, a large elevation in peroxidase levels occurred especially while the bile ducts were becoming enlarged. One might postulate that increased aldehyde and ketone production occurred to a limited extent at this early stage of infection. Catalase was not significantly suppressed. Within six months after infection the hepatic peroxisomal and transaminase enzyme levels had returned within normal range.

In summary, it appears the major changes in the liver parenchyma occurred within the first few months of infection. The capacity of the liver to regenerate cells was apparently unaffected during the course of infection and the minor variations in the hepatic enzyme levels soon returned to normal during the early stage of infection. The increase in

peroxidase might have been in response to products released by the damaged bile ducts.

A wide range of susceptibility to P. concinnum infection and hence the number of flukes which initially became established during experimental infection was noted. Because sampling happened to select cats with lower initial infections, the number of flukes recovered at necropsy from each cat appeared to decrease during the first six months of study; however, this was not the case at nine months. A self-cure mechanism does not appear to be involved in this disease. Two cats in the long term experiment (Group E) have passed ova for over 18 months after the initial experimental infection, thus the life span of the parasite is well over one and one-half years.

Bile samples collected from cats during the chronic stage of infection (after the first four months) revealed interesting data concerning this host-parasite relationship. The concentration of several of the natural components found in feline bile were elevated in parasitized cats. It is possible the flukes accelerate some of the natural degradative processes which normally occur in bile. An attempt is made to identify the compounds found in feline bile but it is highly probable that one of the proteins found to be elevated in parasitized cats is a mucoprotein derived from the bile duct epithelial lining which was mechanically pulled off the bile duct by the fluke. This event is known to take place in human clonorchis infections (Hou, 1955).

One protein however in the bile of cats infected with liver fluke was successfully identified in this study as an immunoglobulin produced by the host in direct response to the P. concinnum infection. The

immunoglobulin, IgG, produced by the host, apparently coated the parasite in an attempt to eliminate or destroy the fluke. However, it appears the antibody was insufficient to complete this task. Other parasites have been shown capable of binding host immunoglobulins to their surfaces; this is the first case where a digenetic trematode was shown conclusively to bind host specific IgG. Immunoglobulins bound to the surface of parasites may function as blocking antibodies (Lewert, 1970). That is, the host protein coats the surface of the parasite and thus somatic antigens of the fluke are no longer exposed to the immune system of the host. This is an apparent protective mechanism for many parasite which live within the body, like Toxoplasma (Araujo et al., 1974). The importance of blocking antibodies in biliary or intestinal parasitism is unclear; further studies are especially needed to elucidate the role of blocking antibodies in platynosomiasis.

The concept of antigenic mimicry or shared antigens does not apply in the host-parasite system of cat-P. concinnum. Host antigens were not found on the surface of the parasite. In other words antigens of neither feline liver, serum proteins other than IgG, nor red blood cells were located on the surface of the fluke. Apparently the parasite does not require shared host antigens in order to survive within the host. Other hepatic trematodes have also failed to show antigens shared in common with the host (Hughes et al., 1972, Taylor et al., 1974).

An attempt was made to assess the role of cellular and humoral immunity in feline platynosomiasis. Cats have two subpopulations of lymphocytes, those which are thymus dependent (T cells) and those which are bone-marrow derived (B cells). B cells have surface immunoglobulins

and complement receptor sites on their surfaces. Cat T lymphocytes have receptor sites for rodent and guinea pig erythrocytes (Taylor et al., 1975). Thus T and B lymphocytes in cats can be differentiated by their membrane receptor sites. The number of T and B lymphocytes in parasitized and control cats were determined: about 40% of the peripheral blood lymphocytes of control and Platynosomum infected cats were immunoglobulin producing B cells, while approximately 30% of the blood lymphocytes were T. Cells. In infected cats the number of non-processed cells (null cells) was 6% less than in the control animals. Apparently a slightly larger number of peripheral blood lymphocytes have become committed in infected cats as compared to control animals; however, the 6% differentiated cells were equally distributed between the B and T cell populations.

Although T and B cell assays give valuable information concerning the immune composition of the lymphoid system, it reveals little concerning the ability of these cells to function immunologically. Mitogen studies were therefore initiated. Feline B cells differentiated by blast transformation in response to pokeweed mitogen, while, cat T cells underwent cellular division in response to phytohemagglutinin. Cat T lymphocytes did not appear to respond to concanavalin A mitogen. Not all species react in the same manner to various mitogens. For example, human B lymphocytes fail to respond to lipopolysaccharides (Peavy et al., 1970); however, murine peripheral blood lymphocytes undergo blast transformation in response to this mitogen (Greaves et al., 1972). The failure of cat T lymphocyte to respond to concanavalin A is therefore attributed to this sort of species variation and not to a specific

defect in the feline immune system. Feline cells do not fair well in tissue culture with the conditions used. Various technics and modifications were tried but to no avail. Mitogen stimulation is therefore either naturally very low in feline lymphocytes or the optimum conditions for blast transformation have yet to be achieved. Nevertheless the response of feline lymphocytes to mitogen stimulation does not appear to be affected by parasitic infection. The inability to induce blast transformation of sensitized cells with cat liver fluke antigens was disappointing. Further studies using other tissue culture regimes may prove adequate for assessing the functional role of T and B lymphocytes in response to P. concinnum infection.

The susceptibility of cats to reinfection was examined. Naturally infected cats were experimentally reinfected with P. concinnum infective stages. Three or eight weeks later the cats were euthanised, necropsied, and the number of adult flukes from the primary, and the number of juvenile flukes from the secondary infection present in the liver, were counted. The number of juvenile flukes recovered from the challenge infection was compared to other cats receiving the same dose as a primary infection. The results showed that the number of new parasites established in naturally infected cats were not substantially reduced. Thus, previous infection did not seem to offer protective help against reinfection in cats which had a low initial parasite burden. However, in one cat which had a large parasite load initially the number of secondary flukes recovered was smaller. Although it appeared there was a reduced susceptibility to secondary infection, this phenomenon was probably regulated more by the availability of space within the bile

duct (i.e., juvenile flukes had to compete with the already established adults for living space and nutrients) than by an immune mechanism. This concept has been proposed in fascioliasis (Sinclair, 1973).

The role of cell mediated immunity in the establishment of infection was assessed in an *in vivo* study. The results were most interesting and promising. The transfer of serum from a cat with liver flukes gave no passive protection to the recipient when challenged with *P. concinnum* infective stages. The protective value of serum has been questioned in a number of parasitic infections (Chu *et al.*, 1971, Sun *et al.*, 1969). In fact the passive transfer of serum is often detrimental to the host (Pierce *et al.*, 1963, Kassai *et al.*, 1967). It is possible the blocking antibody present in the passively transferred serum from infected cats helped perpetuate platynosomum infection.

Kittens which have received sensitized lymphocytes from a *P. concinnum* infected donor had 58% reduction in fluke burden as shown at necropsy. Corba *et al.* (1971) reported 65% reduction in *Fasciola hepatic* fluke burden in a calf which received sensitized lymphocytes from a homozygous twin donor prior to challenge. Comparable results to those in this dissertation were thus obtained in these two parallel experiments studying biliary digenetic trematodes.

Cell mediated immunity probably plays an important role in the regulation of the early disease processes of platynosomiasis, especially in cats with pathologically normal bile ducts. T and B lymphocytes were transferred from the donor cat to recipient animals. The B cells probably had become sensitized to cat liver fluke antigens during the primary infection. The massive cellular infiltration of the lymphocytes

around the newly parasitized bile ducts would result in the presence of plasma cells in this area. Plasma cells would produce antibodies almost directly into the biliary tract. Although IgM has not been studied, it is highly possible the release of IgM into the bile had anti-fluke activities.

The mechanism whereby the T lymphocytes function to decrease the fluke burden is poorly understood (Larsh et al., 1966, Armour et al., 1974). T cells have a variety of functions. They are intimately involved in the chemotaxis of PMN, and other lymphocytes into an area of damage. Perhaps T lymphocytes in response to cat liver fluke antigens release various lymphokines such as migration inhibition factor (MIF) into the blood and keep the macrophages from migrating out of the area (Dumonde et al., 1969).

The transfer of T cells which have been sensitized to P. concinnum may contain another important lymphokine, that of transfer factor. Although transfer factor has not been isolated from felines, its role in liver fluke infections may prove to be interesting (Edynak, 1975).

At this time the importance of the roles of humoral and cell mediated immunity cannot be determined with certainty. Serum from chronic infections confers no protection and may be detrimental to the host as cats receiving the passive transfer of immune serum had the largest number of parasites. The parasites were of large size and the pathology around the bile ducts was generally more pronounced than the other two groups of animals in this comparative study. In cats receiving cells prior to primary challenge, however, the number of parasites was reduced, the size of the parasite was markedly smaller, and the flukes

showed a delay in their developmental sequence.

The hematologic picture of the three groups of cats was also interesting. Untreated and lymphocyte transfer cats showed a very minor increase in the serum transaminase levels. However a substantial eosinophilia was present. In the serum treated cats a large increase in serum transaminase levels was present denoting liver damage. On the other hand, the numbers of eosinophils in the serum remained close to preinfection levels. The role of eosinophilia in helminthic infections has not been elucidated (Mahmound et al., 1971). Cattle which have a thymic deficiency did not show the eosinophilia during Fasciola hepatica infection as do normal immune-intact animals (Flagstad et al., 1972). In these animals the number of flukes recovered after challenge was also noticeably higher. Parasitic diseases and allergic reactions trigger an eosinophilic response and the production of reaginic antibody (Jarrett, 1973). In a pilot study the IgE level (i.e., an immunoglobulin in the serum of cats which cross-reacts with anti-human IgE) was higher after infection than prior to infection in cats with platynosomiasis. The relationship between eosinophilia, IgE, and the cellular immune response should provide important answers regarding the immune mechanism of the host-parasite association (Cohen, 1974).

In summary the host immune system is active in platynosomiasis both at the humoral and cellular levels. Plasma cells infiltrate around the bile ducts in response to infection. The pathology seen is characteristic of an in situ antibody (Cordy, 1970). Perhaps IgM is initially produced and is important in the early stages of the infection, but IgG is soon produced which serves as blocking antibody and is eventually

advantageous for the parasite. Mechanical irritation and metabolic products of the parasite induce fibrotic connective tissue to form around the bile ducts. Thus the host "walls-in" the parasite and some of its metabolic products. An equilibrium is established between the host and parasite throughout the chronic stage of platynosomiasis.

In this dissertation many aspects of the host-parasite relationship between the liver fluke Platynosomum concinnum and its natural dininitive host the domestic cat, Felis catus, have been examined in order to evaluate the veterinary significance of this infectious feline disease. The major damage to the liver, gall bladder and bile ducts takes place during the prepatent period of the disease. Prior to the release of ova in the feces, an elevation in serum transaminase and total bilirubin levels may occur. Elevated transaminase levels due to platynosomiasis could easily lead one to misdiagnose the condition in the cat as hepatic carcinomas, feline viral hepatitis or undetermined biliary obstruction.

The severity of the disease and the fluke burden cannot be determined from hematologic and serum biochemical data. Elevated serum transaminase enzyme levels are not pathognomonic for platynosomiasis since during the chronic stage of infection the serum levels of these enzymes are within the normal range for cats. The low grade eosinophilia present in cat liver fluke infections is also a characteristic of many other common parasitic infections.

The number of ova present in the fecal sample is also of little value to the veterinarian trying to assess the extent of the disease in his feline patient. The number of ova produced are apparently

higher in the beginning of infection than later in the disease. An average of 1-100 ova per gram of feces per cat liver fluke present has been estimated. Thus the recovery of 20 ova per gram of feces could represent 20-2,000 parasites in the liver. In a random sample of infected cats the serum transaminase levels were normal; as were the hepatic transaminase and peroxisomal enzyme levels. Thus a cat with chronic platynosomiasis has an almost normal clinical, hematologic, serum-biochemical and hepatic enzyme profile.

The similarities between feline platynosomiasis and human Clonorchis sinensis infection are striking. The two flukes are morphologically similar, their route of migration up the common bile duct and patterns of establishing themselves in the gall bladder and biliary tract are apparently identical. Clonorchis sinensis matures more rapidly leading to a shorter prepatent period than that seen in P. concinnum infections (Brown, 1964). The clinical signs and symptoms of the two diseases are similar with apparent good health being the predominant characteristic with low dose infections and epigastric problems noted in high dose infections (Cheah et al., 1970). The pathologic findings are apparently similar; however, there is considerable confusion in the literature concerning the extent of fibrotic involvement in C. sinensis infection. The cat has been considered a good animal model for various human diseases. The Platynosomum concinnum-Felis catus host-parasite relationship is proposed here as a suitable animal model for human clonorchiasis studies.

A D D E N D U M

Studies on Natural and Experimental Definitive Hosts other than the Domestic Cat

Introduction

The domestic cat is the principal host of P. concinnum; however, the wild cat Herpailurus y. yaguarondi (Barbosa et al., 1949), the ferret, Gieson vittata (Pinto et al., 1935), and the opossum Didelphis marsupialis (Eckerlin et al., 1962) are also found naturally infected in the wild. The mouse, Mus musculus (Maldonado, 1946) and the spotted skunk Spirogale putorius ambarralis (Eckerlin et al., 1962) were shown to be adequate definitive hosts of experimentally induced Platynosomum concinnum infections.

A search for another animal species which might serve as a definitive host was undertaken. In Hawaii the mongoose, Herpestes auropuntatus, was considered as a possible naturally infected reservoir host for P. concinnum. This nocturnal omnivore shares the same general habitat as cats. Various liver lesions of undetermined etiology have been reported at necropsy in the mongoose, although flukes had not been found (Palumbo, 1973). Fifty feral mongooses were captured and examined for the presence of P. concinnum.

Rodrigues (1963) reported that the bird liver fluke, P. illiences and the cat liver fluke, P. fastosum (syn. concinnum) were morphologically similar enough to be considered as the same species. This is probably an incorrect conclusion; however, if further studies on the biology of Platynosomum spp were to demonstrate otherwise, then various

avian species would also serve as definitive hosts of P. concinnum.

A study was also initiated to find a laboratory animal which might serve as an experimental model for P. concinnum infection. Viable metacercariae were therefore orally administered to the Wistar rat (Rattus rattus), the mongoose (H. auropunctatus), the quail (Coturnix japonica) and various strains of the common laboratory mouse (Swiss White, DBA, and Black 6).

Materials and Methods

Method for surveying naturally P. concinnum infected mongooses: Fifty feral mongooses were donated to the Department of Comparative Medicine within four days of capture. The mongooses were euthanized and necropsied. Gross pathologic observations were made of the liver and other organs. The livers were excised, minced in saline and examined with the aid of a dissection microscope for the presence of P. concinnum adult flukes as well as immature stages that might be present. A bile sample, collected directly from the gall bladder with a needle and syringe was examined (microscopically) for the presence of ova.

P. concinnum experimental infection of laboratory animals: Metaceraciae were removed from the biliary tract of Bufo marinus, counted and orally administered via stomach tube to various laboratory species. Table 16 shows the number of animals studied and the infective dose of metacercariae each received. Blood and fecal samples were collected periodically during the course of experimental infection for the mongoose study and immediately prior to euthanasia for the

mouse, rat, and quail study. Standard hematologic and serum biochemical methods, as described earlier were used. Animals were euthanised and necropsied according to the time schedule presented in Table 16. The livers were diced and teased in saline and then examined for the presence of P. concinnum with the aid of a dissection microscope. Fecal samples were collected and examined for the presence of ova using the formalin-ether technic.

Small sections of murine hepatic tissue were stored frozen and subsequently analyzed for the activity level of several peroxisomal enzymes.

Table 16

Infection and Necropsy Schedule
for Possible Definitive Hosts

Species	No. & Sex	Oral Infective Dose P. concinnum Metacercaria	Euthanasia Time (Days) Post-Infection
Rat			
Rattus rattus	2 M	+300	70
Mangoose			
H. auropunctatus	4 M	+300	18,45,52,59,66,
	4 F	+300	69,80
	4 C*	0	
Quail			
Coturnix japonica	4 M	125	
	4 F	125	70
	4 C*	0	
Mice			
Mus musculus			
Swiss white	50 M	25	1,3,4,5,6,7 & wkly. to 72 da.
DBA	21 M	25	Wkly. 1 - 72 da.
Black 6	2 M	25	70

*Control, equal number male and females

Results

Observations on the susceptibility of rats and quail to P. concinnum infection: Flukes were not recovered from rats and quail orally administered infective stages of P. concinnum. The livers were normal; no signs of Platynosomum infection were present. Fecal examination revealed no ova at any time during the experiment.

Studies on the susceptibility of naturally and experimentally infected mongooses: The fifty feral mongoose livers examined presented varied pathology but enlargement of bile ducts, so characteristic of platynosomiasis, were not seen. Flukes were not recovered from any of these livers. Neither fecal nor bile samples were positive for P. concinnum ova. All feral mongooses were therefore deemed P. concinnum negative.

Flukes were recovered from one of the eight experimentally infected mongooses, representing a 12.5% infection rate. The other seven animals showed no pathologic signs at necropsy such as enlargement of the gall bladder or dilatation of the bile ducts attributable to P. concinnum infection. These seven animals had not become infected following oral administration of the metacercariae. The hematologic and biochemical profiles of all eight mongooses remained within the normal range (Palumbo, 1974b) during the period of study. Fecal samples were examined and were routinely negative for P. concinnum ova; however, cociddia oocysts were present throughout the study. These coccidian oocysts were part of the animals normal intestinal flora.

The two flukes recovered 42 days post-infection from the one mongoose were morphologically identical to those recovered from cats at

an equivalent period of time after infections.

Experimental infection of mice: 43.8% of the Swiss white and 53.3% of the DBA mice were successfully infected with platynosomiasis. No flukes were recovered from Black six mice. The infected mice had fluke burdens ranging from 1--6 or 4--24% of the original experimental infective dose.

Hematologic and biochemical studies of Swiss white mice were made at necropsy. The hematologic data together with the fluke burden found in each animal at necropsy is presented in Table 17. A slight leucocytosis was noted 4.5--7 weeks post-infection. Serum glutamic oxaloacetic acid transaminase enzyme activity levels were slightly elevated; however, wide variations are normally measured in mice due to their stress response to aneathesia and handling prior to euthanasia. An eosinophilia was not seen. All other hematologic and biochemical values were within the normal range for mice.

The developmental stages of the flukes recovered at necropsy are pictured in Figure 49. The initial growth rate of the fluke in the mice biliary tract was retarded compared to the growth rate of the fluke in the cat. However, once the fluke had become established, it developed at a slightly faster rate and reached a longer length in the mouse by 45 days than in the cat. A rudimentary cirrus sac was present in developing mouse flukes 21 days after infection; this structure had not appeared by day 26 in flukes recovered from cats. Flukes established in DBA mice seemed to have a retarded rate of development when compared to those of Swiss white mice.

Peroxisomal liver tissue enzymes activity levels were analyzed in

TABLE 17

HEMATOLOGIC FINDINGS OF SWISS WHITE MICE INFECTED WITH P. CONCINNUM

DAYS POST-EXPERIMENTAL INFECTION:	0	7	14	21	33	42	49	57
FLUKE BURDEN:	0	1	1	3	1	2	6	3
PCV (%)	40	50	42	45	52	52	50	42
Icterus Index	3	3	5	3	5	3	3	5
Sedimentation Rate @ 15 min.	0	0	0	0	0	0	0	0
30 min.	0	0	0	0	0	0	0	0
45 min.	1	0	1	1	0	0	0	0
60 min.	2	0	1	1	0	1	1	0
BUN (mg/100 ml)	10	15	20	15	25	30	15	30
Glucose (mg/100 ml)	120	120	110	130	130	90	120	130
Total Protein (gms/100 ml)	4.8	5.8	5.0	4.2	5.4	6.0	5.0	5.6
Hb (gms/100 ml)	-	16.8	-	-	16.6	-	15.0	15.0
WBC/cmm	10,800	7,600	8,000	6,800	14,200	10,500	9,200	8,100
SGOT (SF Units)	-	76	-	-	100	-	48	38
SGPT (SF Units)	-	55	-	-	28	-	14	40
DIFFERENTIALS (%):								
Eosinophils	0	0	0	0	2	0	0	0
Basophils	0	0	0	0	0	0	0	0
Juvenile Neutrophils	0	0	0	0	0	0	0	0
Band Neutrophils	0	0	0	1	0	0	0	1
Segmented Neutrophils	55	55	36	32	22	7	10	31
Lymphocytes	45	44	63	66	74	92	90	66
Monocytes	0	1	1	1	2	1	0	2

FIGURE 49

THE DEVELOPMENTAL STAGE OF PLATYNOSMUM CONCINNUM IN THE SWISS WHITE MOUSE

A. Infective stage (metacercaria) isolated from the liver of the toad (Bufo marinus).

B. Juvenile fluke recovered from the mouse 7 days post-infection.

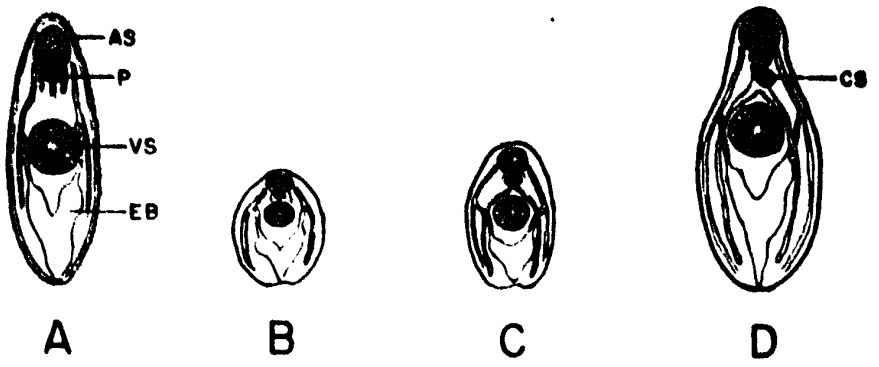
C. Immature fluke 14 days post-infection.

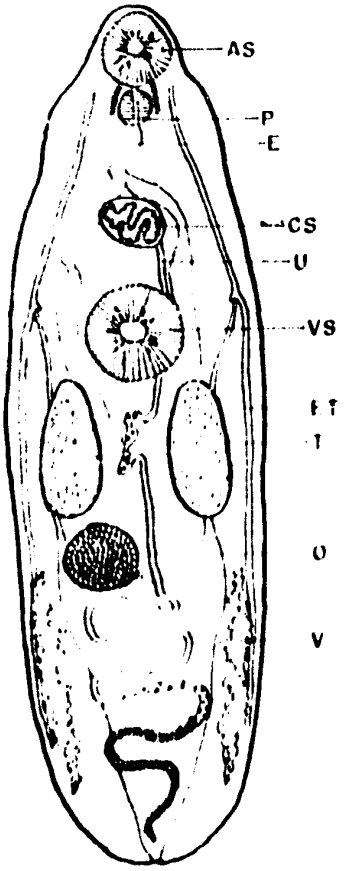
D. Maturing fluke 21 days post-infection.

E. This fluke is mature 33 days after infection.

F. 42 days post-infection

AS, anterior sucker; C, caeca; CG, cephalic gland;
CS, cirrus sac; E, esophagus; EB, excretory bladder;
ET, excretory tubules; MG, Mehlis gland; O, ovary;
OO, ootype; P, pharynx; SR, seminal receptacle; T, testes;
U, uterus; V, vitelline gland; VS, ventral sucker.





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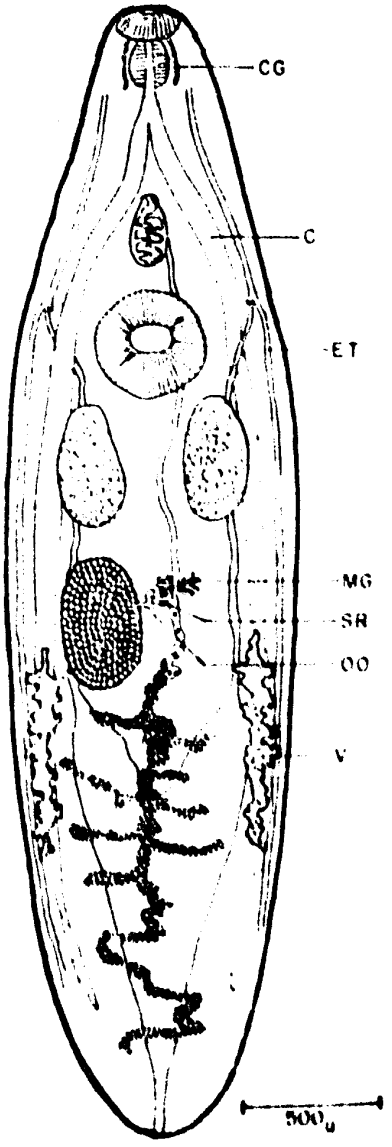

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normal as well as in experimentally infected Swiss white mice. The data are shown in Table 18. A 96.7% elevation of both DAAO enzyme activity level and a 94% uricase enzyme level was observed in parasitized mice. All other peroxisomal enzyme levels remained unaltered. No statistical correlation was found between liver tissue enzymes levels elevations and parasitic burden found at necropsy.

Ten weeks after infection ova were not detected in fecal samples collected from Swiss white mice; however, ova were present in feces of DBA mice 56 days after infection.

Discussion and Conclusions

The host specificity of P. concinnum infection was examined in four species other than the domestic cat. In rats, no definite conclusion concerning its lack of susceptibility to P. concinnum infection was made in this study because of the limited number of rats studied. One might speculate however that the absence of a gall bladder in the rat would result in a different bile concentration as compared to the cat and might provide an insufficient chemotactic gradient for the juvenile fluke as it migrates from the intestinal lumen toward the biliary tract.

The Japanese quail also was not successfully infected experimentally with P. concinnum although the infective oral dose administered and the number of birds studied were adequate to properly evaluate the susceptibility of this species. Some authors have maintained that P. illiences is synonymous for P. concinnum. Although most birds parasitized by P. illiences are carnivores as the broad-winged hawk

TABLE 18

PEROXISOMAL ENZYME ACTIVITIES FOR CONTROL AND PARASITIZED SWISS WHITE MICE

		nM H_2O_2/mg Protein N				
		DAAO	OHAO	URICASE	PEROXIDASE	CATALASE K/N*
CONTROL		0	331.9	131.8	131.8	64.4
		35.1	326.1	30.7	83.3	118.1
		11.9	191.3	28.9	95.6	128.3
		75.2	387.9	103.7	81.1	156.9
		00.0	437.9	178.8	79.6	153.9
		-	380.0	36.5	58.4	66.1
AVERAGE ± S.D.		24.4±31.8*	342.5±84.6	85.1±62.9	88.3±24.5	114.6±41.0
P. concinnum POSITIVE						
Days Post Infection	No. Flukes/ Mouse					
7	1	95.3	743.5	74.1	83.3	118.1
14	1	69.4	387.9	248.3	126.9	132.8
21	3	19.1	286.9	171.7	76.2	113.2
33	1	19.0	238.6	126.9	99.1	120.0
42	2	74.4	475.0	213.9	137.4	200.0
49	6	51.8	378.3	216.7	98.3	116.0
57	3	7.1	177.3	106.4	14.3	75.7
AVERAGE ± S.D.		48.0±33.5	383.9±187.5	165.4±64.8	90.8±40.3	125.1±37.4

*Average ± 1 Standard Deviation

(Buteo platypterus), the barn owl (Tyto alba), the roadside hawk (Rupornis magnirostris), other resemble the Japanese quail in their food habits as Ramphastus sp. and Rupicola sp. (Berger, 1974, Yamaguti, 1958). The insusceptibility of C. japonica to P. concinnum infection was not unexpected with respect to our current knowledge of Platynosomum biology.

The mongoose, a member of the family Carnivoridae, apparently has a low susceptibility to Platynosomum infection as only one of eight mongoose was successfully infected experimentally. In addition, naturally infected mongooses were not found. Environmental and dietary factors may help explain why this animal, shown experimentally to be susceptible, does not have more naturally infected cases in the wild.

Although the mongoose and the cat (especially the cat which has reverted to a semi-feral state) share many habitats, the mongoose in general is distributed in more rural areas while the stray or feral cats tend to remain near or in inhabited areas. The incidence of infective stages in intermediate hosts such as the skink, gecko, and bufo toad are generally higher in these urban areas (Sinclair, 1974). In addition, while cats are notorious hunters who seem to relish geckos, it does not appear that the mongoose, despite scavenger and predatory characteristics, utilizes these sources as food.

The mouse was successfully infected experimentally with P. concinnum in this study. Maldonado (1945) stated that mice (probably Swiss white mice) were susceptible and that the developmental sequence in mice was probably similar to that of the cat. The data presented

in this study basically supports this conclusion. However, the initial developmental stages in the mouse appeared to be more rapid than in the cat. Well developed ovary and testies were present by 21 days post-infection in mice; these structures developed about 10 days later in the cat. The reason for the more rapid initial development in the mouse than in the cat is not known; however, usually fewer flukes were present within the biliary tract of the mouse. Perhaps more nutrients are available per fluke and the lack of competition for space allowed for quicker maturation of the parasite. The developmental sequence described by Maldonado for P. fastosum in mice is similar to our findings; however, the growth rate and general size of the flukes described by Maldonado were larger than those observed in this study.

During the course of experimentally induced P. concinnum infection in the Swiss white mouse, hematologic and serum biochemical values were analogous to those noted in cats infected with low doses of metacercariae. Eosinophilia and elevation of serum tansaminases enzyme activity levels were moderate at best.

The effect of P. concinnum on the liver parenchymal tissue peroxisomal enzyme activity levels of mice differed from that of the cat. Both DAAO and uricase levels were elevated in mice; peroxidase was not elevated as it was in the cat. Platynosomum infection apparently triggered augmented hydrogen peroxide production in mouse hepatocytes as a result of increased DAAO and UO activity. Hruban et al. (1969) reported that mice were deficient in the DAAO enzyme, but the modern method of Hokama et al. (1973) was able to detect these low levels of

enzyme activity.

In conclusion, both the mongoose and the mouse can serve as definitive host animal models in the laboratory for the study of P. concinnum. These preliminary studies indicate that the mongoose has a low susceptibility to experimentally induced infections but the Swiss white mouse can easily be infected. In addition, hematologic, serum biochemical, and developmental studies of the parasite suggest the mouse in particular is a convenient laboratory animal to study feline liver fluke disease.

PLATE 1

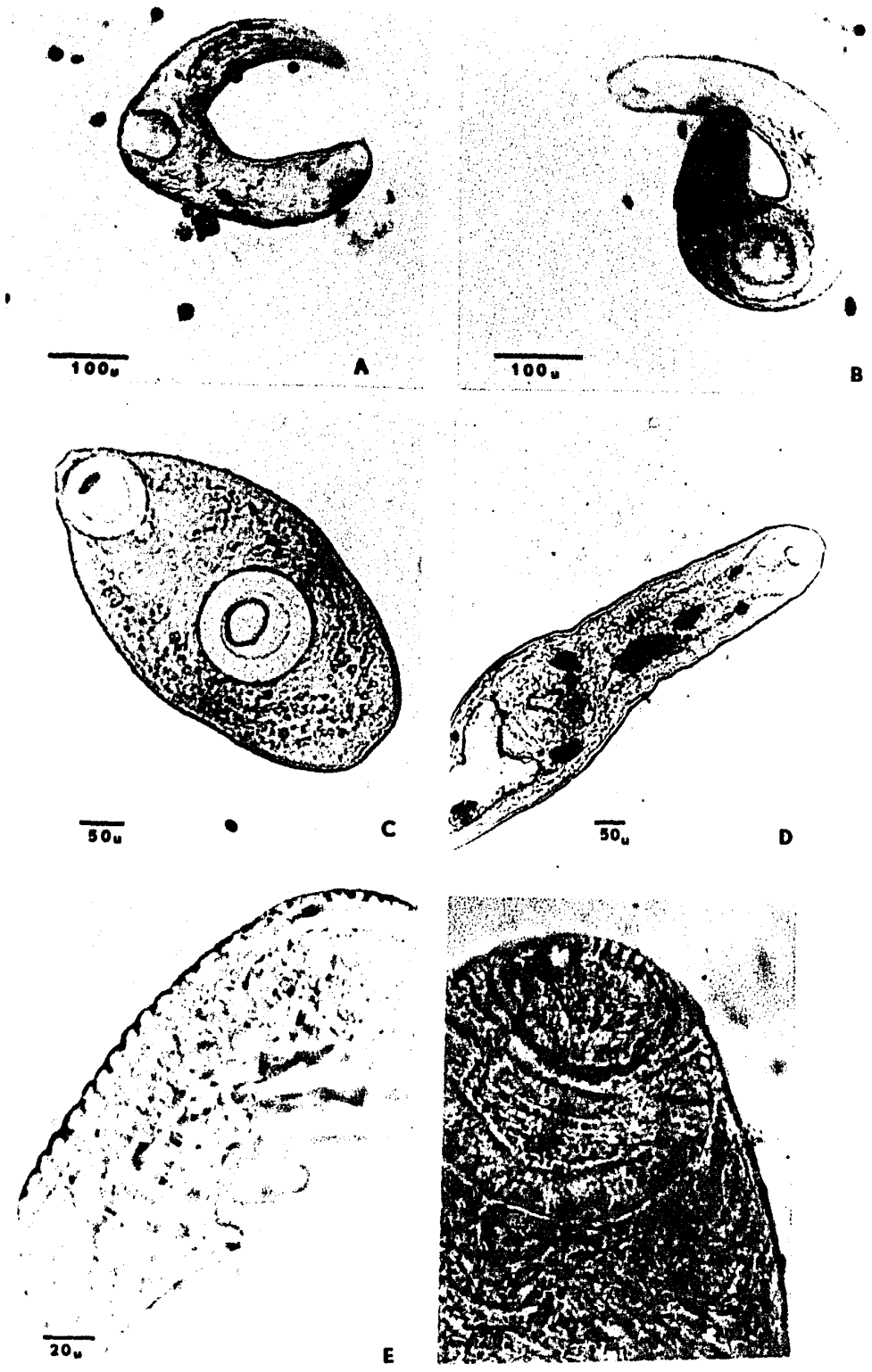
THE INFECTIVE STAGE OF PLATYNOSOMUM CONCINNUM RECOVERED FROM THE LIVER OF THE TOAD, BUFO MARINUS

A-B. The infective stages are actively motile and pleomorphic when removed from toad liver.

C-D. This stage may contract into a shape characteristic of the adult form of P. concinnum (C) or "stretch" by extending the distance between the two suckers (D).

E. The thick cuticle with indentations on the surface which probably aids in protecting the fluke during infection.

F. The anterior sucker ridges probably aid the infective stage grasp to surfaces while migrating up the common bile duct.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A
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B
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C
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D
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E

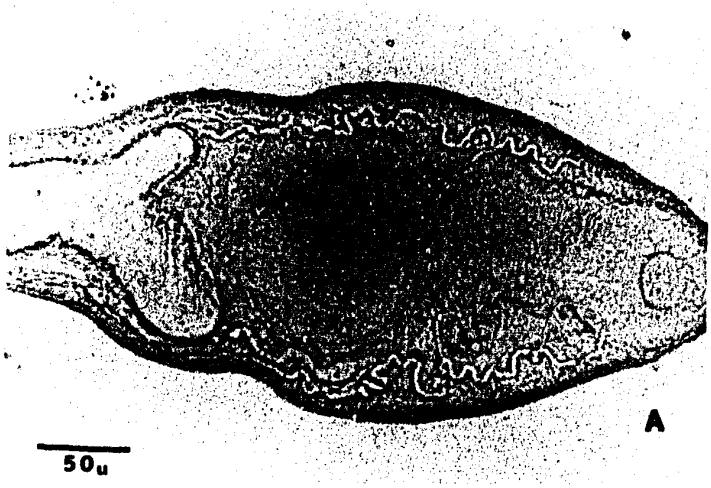
PLATE 2

JUVENILE FLUKE RECOVERED FROM THE LIVER OF A CAT SEVEN DAYS AFTER INFECTION

A. The parasite has decreased in length while increasing in width. The morphology of the juvenile fluke is the same as that found in the infective stage.

B. Microvilli cover the surface of the fluke.

C. Ridges and rasping hooks are present in the acetabulum for feeding and holding position in the bile ducts.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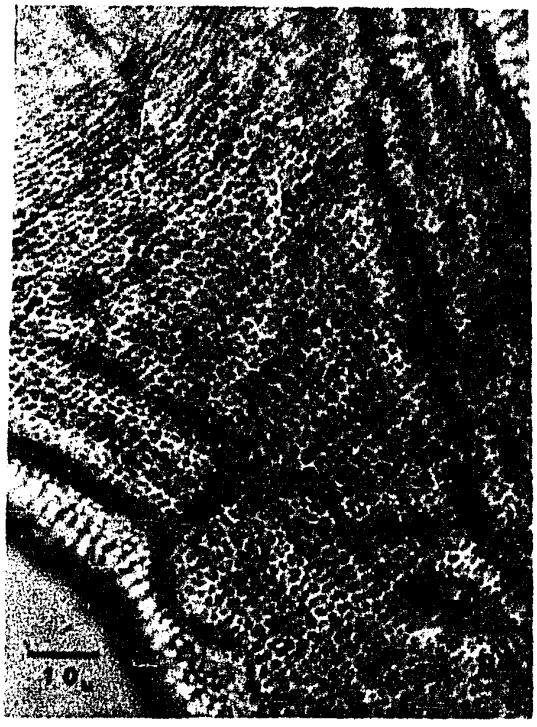
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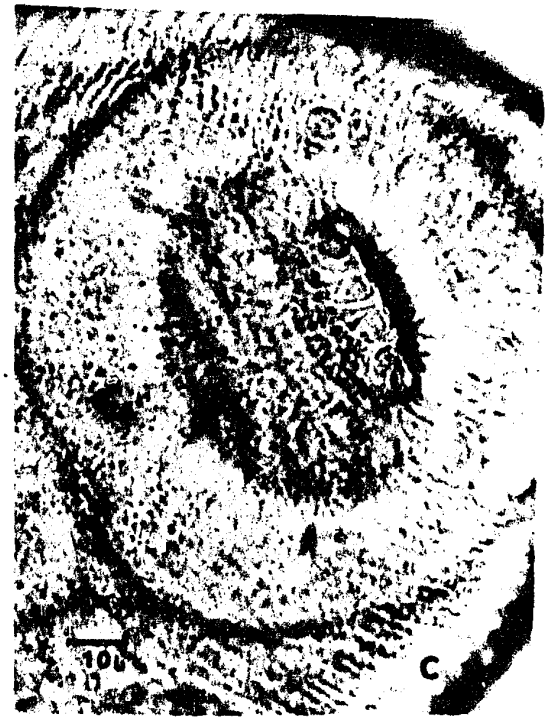
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PLATE 3

MATURING FLUKE 19 DAYS AFTER INFECTION

The anterior sucker (AS) and ventral sucker (VS) are prominent features. The beginning of an oviduct (O) can be seen.

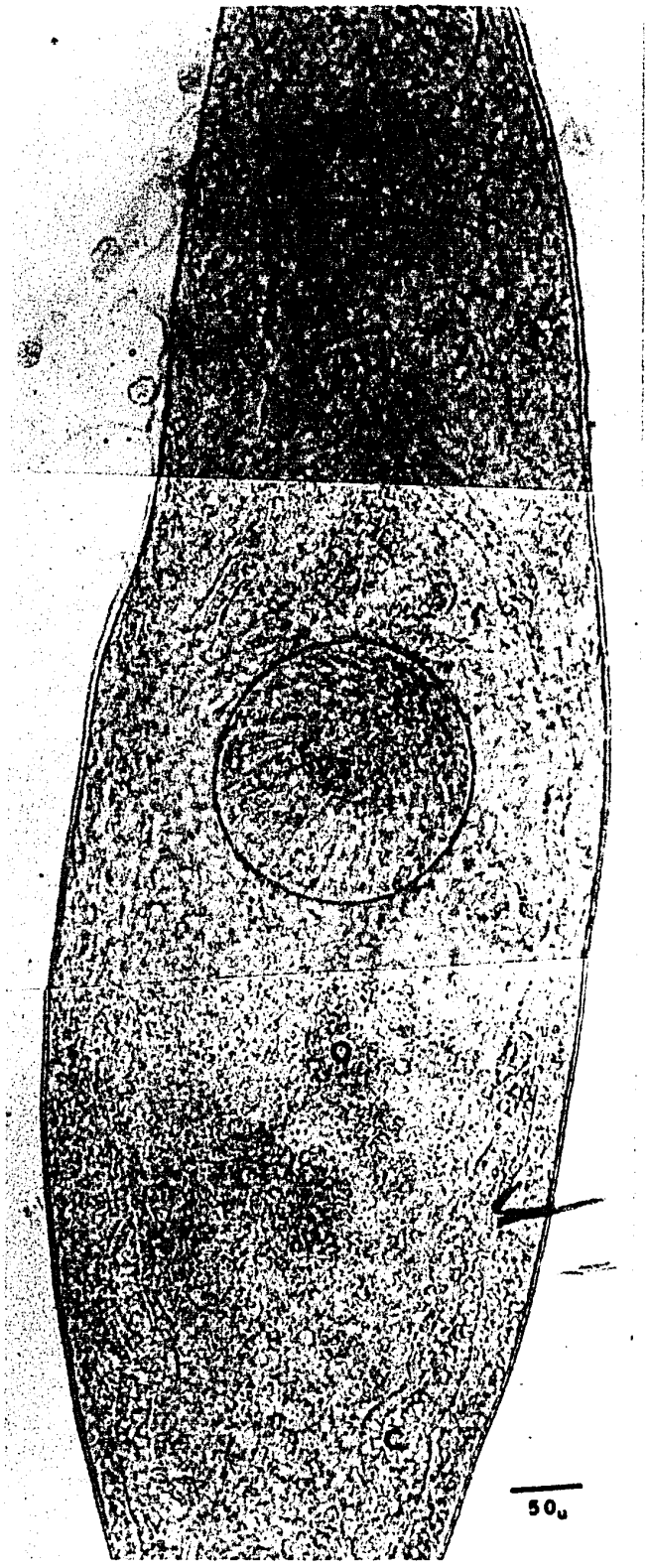
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PLATE 4

THE DEVELOPMENTAL STAGE OF THE FLUKE 21 DAYS AFTER INFECTION

A. The bifurcation of the excretory tubules occurs at the level of the anterior portion of the ventral sucker (VS).

B. The muscular pharynx and the esophagus branching into the caeca (C) are clearly seen. Cephalic glands are still present.

C. Spermatocytes can be seen developing within newly forming testes (outlined area in photo D).

D. The location of the left testis is outlined. The rudimentary oviduct can be seen.

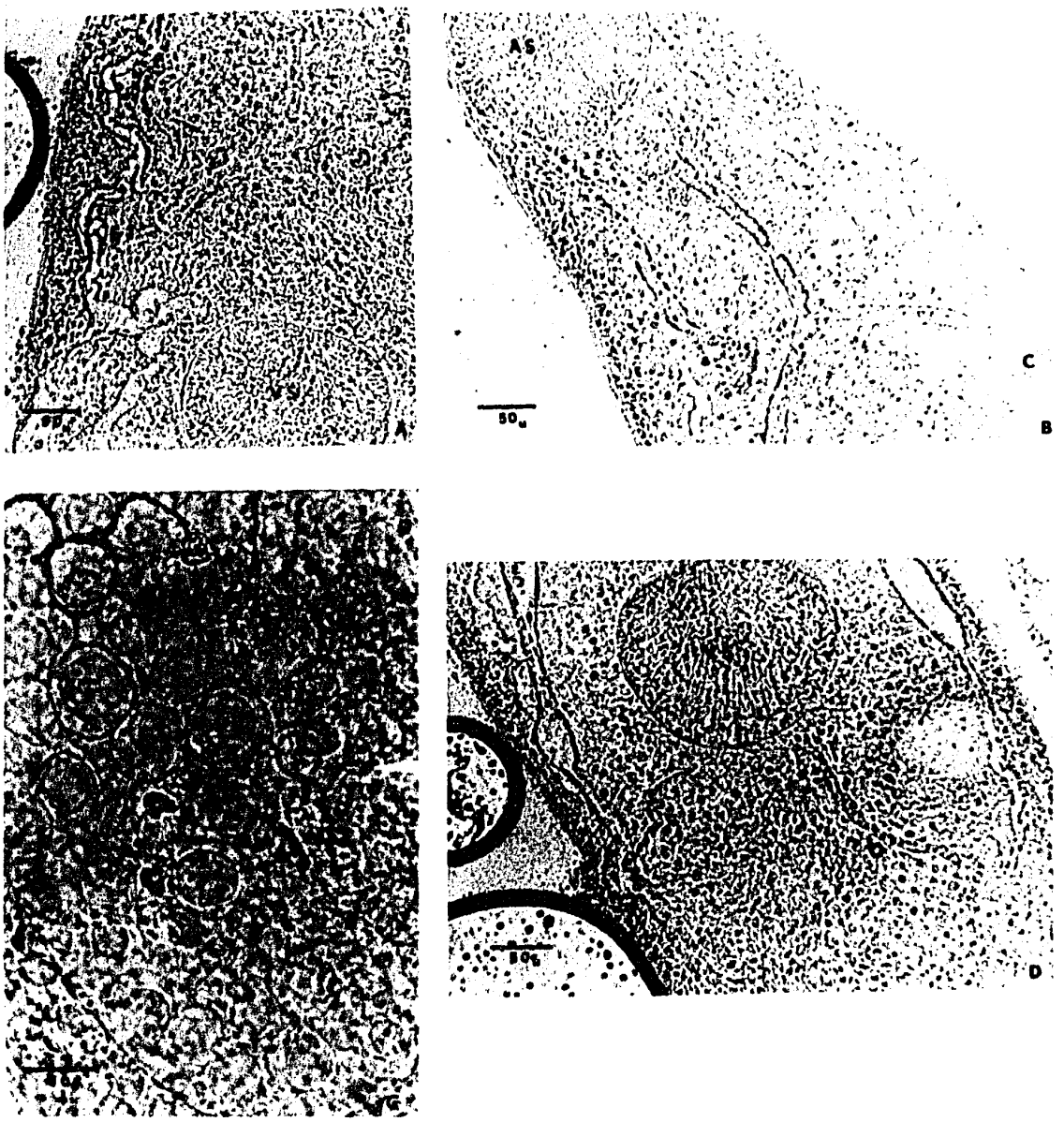

A
B
C
D

PLATE 5

REPRODUCTIVE ORGANS OF PLATYNOSOMUM CONCINNUM

6 WEEKS AFTER INFECTION

A. Both immature ova and vitelline material can be seen in the uterus of the fluke. A testis is pictured on the left of the oviduct with the ovary below. The oval seminal recepticle is located adjacent to the ovary.

B. At greater magnification of the seminal receptacle the mature sperm can be seen.

C. One branch of the seminal receptacle leads to the ovary, the other, to the Mehlis gland.

D. Immature ova can be seen in the oviducts of a few flukes 42 days after infection.

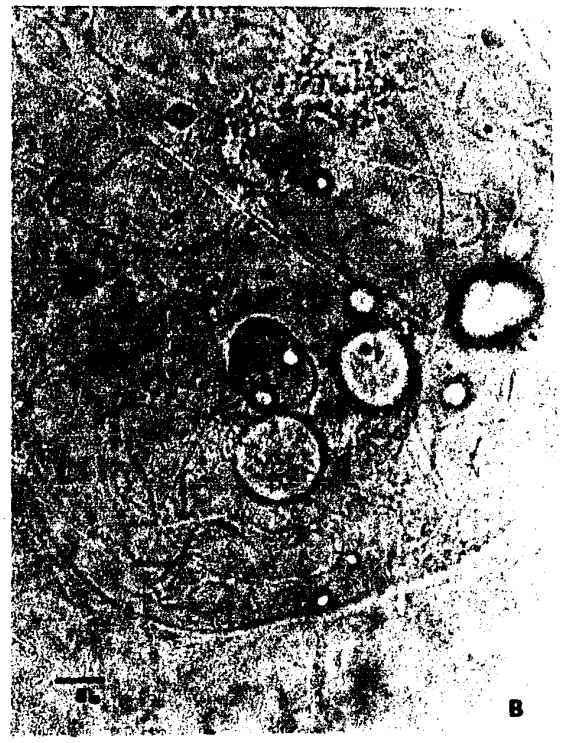


PLATE 6

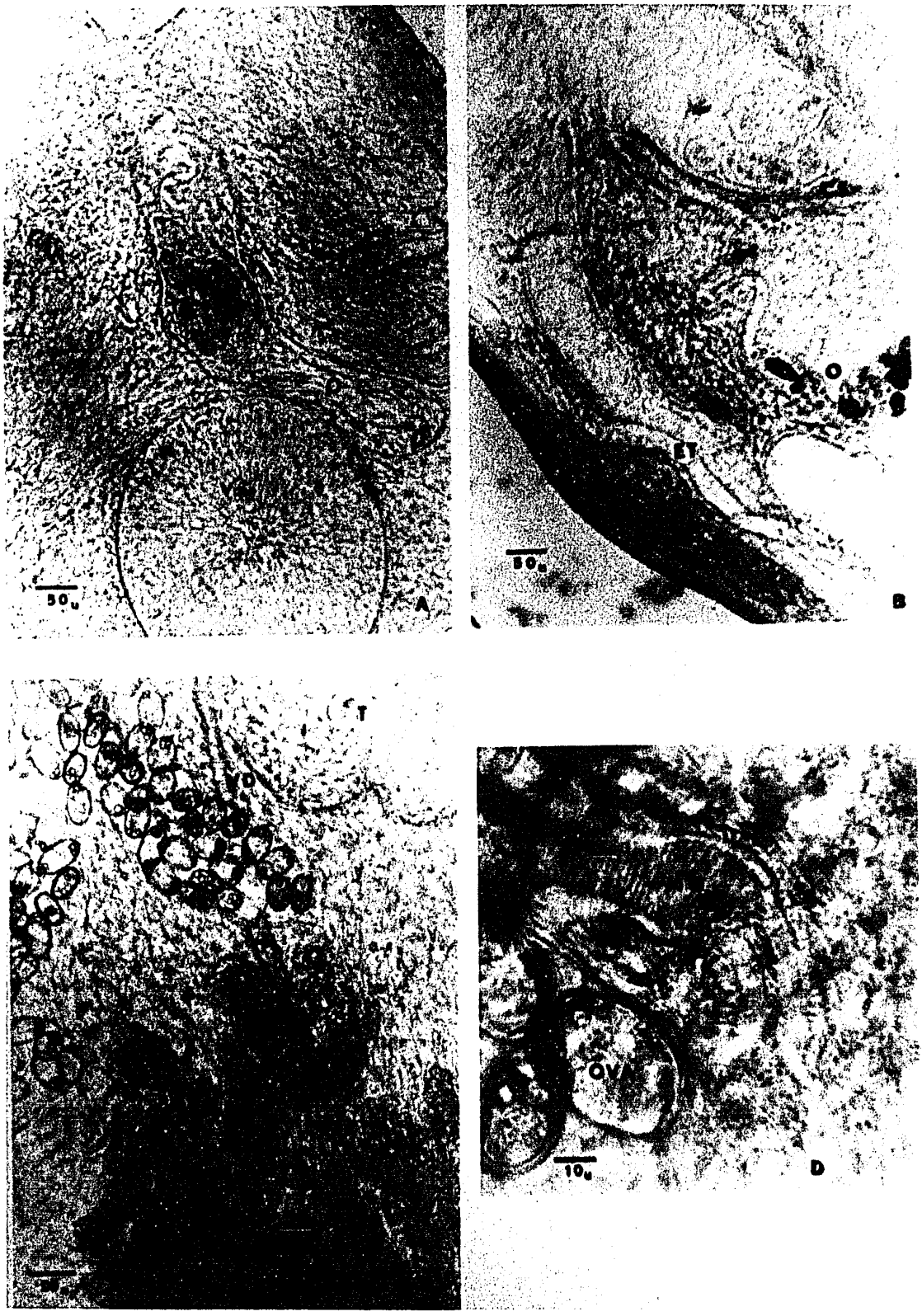
SOME IMPORTANT STRUCTURE OF ADULT P. CONCINNUM

A. The cirrus sac (CS) containing the seminal vesicle and ejaculatory duct is located anterior to the ventral sucker (VS). The oviduct (O) or uterus develops around the ventral sucker (VS).

B. P. concinnum has a common gonadal pore into which the oviduct (O) and the cirrus sac (CS) enters. The excretory tubules are clearly demarcated.

C. By 8 weeks a lacy vitelline gland has formed. The vitelline duct (VD) carries yolk material to the ootype. Embryonated ova are not yet present.

D. The Mehlis gland located in the ootype is well defined and functional by eight weeks.


50μ
A
O
ET
50μ
B
T
O
C
OV
10μ
D

APPENDIX A

TABLE 19

WEIGHT OF INDIVIDUAL CATS PREVIOUS TO INFECTION
IN POUNDS AND OUNCES

CAT #	SEX	WEEKS PREVIOUS TO INFECTION 14	13	12	11	10	8	6	5	4	3	2	5	ESTIMATED INITIAL AVE. WEIGHT
GROUP A														
1	M	8-8	8-0	7-6	7-8	7-6	8-6	8-2	8-6	8-9	8-8	8-7	8-10	8-8
2	M	9-12	8-8	9-0	10-8	9-8	9-8	9-4	9-11	--	--	10-0	10-8	10-0
5	F	--	7-4	--	6-14	6-14	7-0	6-15	6-12	6-14	6-6	6-5	6-5	6-11
6	F	--	5-4	5-8	5-14	5-12	5-6	5-4	5-8	5-6	5-2	5-4	5-5	5-5
7	F	--	7-8	7-6	8-10	8-2	8-8	8-8	8-8	8-6	8-6	8-0	8-0	8-4
8	F	--	--	--	--	--	6-12	6-11	6-15	6-12	6-12	5-12	5-8	6-6
GROUP B														
13	M	7-12	7-8	8-2	7-9	7-14	8-8	8-11	8-11	9-0	8-14	8-12	8-14	8-13
14	M	7-12	7-8	7-2	8-4	8-8	8-2	8-4	8-10	8-15	9-15	8-15	9-3	8-14
15	M	8-0	7-4	7-8	8-2	8-4	8-10	8-8	8-6	8-6	8-12	8-12	8-10	8-10
16	M													
18	F	--	4-12	5-6	5-14	6-8	6-14	6-8	6-12	6-12	7-2	7-0	7-2	7-0
19	F	--	4-8	5-1	4-11	5-8	6-6	6-0	5-12	5-14	6-0	5-10	5-8	5-14
20	F	--	--	--	--	--	7-14	7-4	6-12	7-0	6-14	6-15	6-15	6-15
21	F	--	--	--	--	--	5-4	5-2	5-2	5-2	4-14	4-6	5-0	5-0
GROUP C														
22	M	10-0	9-4	9-4	11-0	8-12	8-10	8-14	8-10	8-10	8-6	8-9	8-10	8-10
23	M	--	--	--	--	6-0	5-12	5-13	6-4	6-9	6-8	6-0	6-0	6-4
24	M	5-4	4-4	5-2	6-8	6-4	6-12	6-14	6-12	7-6	7-8	7-2	7-10	7-6
25	M	5-10	5-8	5-6	6-10	6-6	7-2	7-4	7-1	7-6	7-9	7-4	7-8	7-7
26	F	--	--	--	--	--	6-0	6-6	6-2	6-4	6-0	5-5	6-6	6-2
27	F	--	--	--	--	--	6-2	6-4	6-15	7-6	7-4	7-0	7-4	7-2
28	F	--	8-14	7-13	--	8-12	8-2	8-7	8-9	8-14	9-0	9-4	9-6	9-0
29	F	--	7-0	6-10	--	--	7-12	6-5	6-12	7-2	7-6	7-6	7-8	7-6

TABLE 20

WEIGHT CHANGES IN POUNDS AND OUNCES DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 0	1	2	3	5	6	7	8	9	10	11	12
GROUP A													
1	M	8-3	8-12	8-14	9-0	9-0	9-7	9-6	9-15	9-9	9-13	10-2	10-2
2	M	10-5	10-12	11-0	11-0	11-7	11-7	11-9	11-9	11-9	11-4	11-2	10-13
5	F	6-8	6-10	6-4	6-7	6-10	6-11	6-8	6-8	6-0	6-6	6-8	6-2
6	F	5-7	6-1	6-3	6-0	6-7	6-3	6-12	6-8	6-6	6-8	6-10	6-6
7	F	8-4	8-1	8-4	8-6	8-0	7-14	8-0	8-0	8-0	8-6	8-4	8-8
8	F	6-4	5-11	6-6	6-5	6-6	6-0	6-0	6-0	5-15	6-3	5-8	6-0
GROUP B													
13	M	8-10	9-0	9-3	9-0	9-0	8-12	9-0	9-3	8-12	9-1	9-4	9-0
14	M	9-0	9-6	9-12	9-10	9-10	9-13	9-12	9-6	9-7	9-6	9-12	9-14
15	M	8-11	8-15	9-0	9-4	8-15	9-0	9-2	9-9	9-5	9-2	9-7	9-6
16	M	--	--	--	--	--	--	--	--	--	12-4	12-14	13-0
18	F	7-0	7-3	7-3	7-3	7-11	7-10	7-11	6-2	7-9	7-2	7-12	7-13
19	F	5-11	5-10	5-12	5-11	6-2	5-14	6-0	5-12	5-15	6-0	6-4	6-3
20	F	6-22	7-8	6-14	7-2	6-15	7-1	7-2	7-9	7-0	7-0	7-8	7-6
21	F	4-12	4-12	4-10	5-8	5-0	4-13	4-10	5-0	4-15	5-2	5-15	5-4
GROUP C													
22	M	8-8	8-11	9-0	8-10	9-12	8-10	8-12	8-14	8-9	8-0	8-10	8-12
23	M	6-2	7-0	6-2	6-2	6-7	6-7	6-6	5-15	6-4	6-3	6-3	6-12
24	M	7-8	8-4	8-2	8-2	8-1	8-2	8-6	8-4	8-0	7-7	7-14	8-0
25	M	7-12	8-4	8-0	8-7	9-0	9-14	8-10	8-11	8-14	8-11	8-14	8-12
26	F	6-7	6-14	6-7	6-13	7-2	6-13	7-0	6-15	6-12	6-1	6-5	6-0
27	F	7-3	7-10	7-9	7-9	7-13	8-4	8-3	8-2	7-7	7-15	8-11	8-14
28	F	9-1	9-8	9-8	9-14	9-8	10-0	10-3	9-12	9-2	9-12	9-8	9-4
29	F	7-7	7-14	8-0	8-4	8-5	8-4	8-9	8-5	8-1	8-4	8-4	8-2

TABLE 20 (CONTINUED)

WEIGHT CHANGES IN POUNDS AND OUNCES DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	13	14	15	16	17	18	19	20	21	22	23	24
GROUP A													
1	M	10-2	10-0	10-8	10-9	10-6	10-6	10-6	10-8	10-12	10-12	10-9	10-6
2	M	11-2	11-0	11-0	10-12	10-14	10-11	10-2	11-1	11-2	11-4	11-0	10-9
5	F	6-12	6-15	6-14	6-14	6-8	7-0	6-12	7-2	6-13	6-13	7-0	6-12
6	F	6-13	6-13	7-0	6-12	7-2	6-9	6-14	7-3	6-12	7-3	6-12	6-12
7	F	8-8	8-8	8-10	8-6	8-11	8-5	9-0	9-0	9-4	9-8	9-4	9-4
8	F	5-10	5-8	5-14	5-10	5-8	6-1	5-7	5-8	5-5	5-11	5-6	5-6
GROUP B													
13	M	9-7	9-12	9-12	10-2	9-15	9-10	9-10	10-10	9-14	9-12	10-2	9-12
14	M	9-15	10-1	10-4	9-15	9-14	9-12	9-9	9-7	9-4	9-6	9-7	9-2
15	M	9-9	9-6	10-4	9-13	9-12	9-15	9-11	9-11	9-8	9-8	9-10	9-7
16	M	13-3	13-0	13-5	13-4	13-5	13-4	12-9	12-10	12-8	12-4	11-2	11-10
18	F	7-14	7-14	7-13	7-7	7-11	7-4	7-9	7-15	7-0	6-12	7-0	7-8
19	F	5-15	5-9	6-2	5-8	5-14	5-14	6-2	6-0	6-0	5-10	5-9	5-7
20	F	7-8	7-12	7-14	7-11	7-12	8-2	7-8	7-10	7-4	7-2	7-0	7-4
21	F	5-10	5-10	5-10	5-6	5-10	5-5	5-2	5-0	5-0	5-8	5-10	5-10
GROUP C													
22	M	9-2	9-0	9-8	9-10	9-8	9-4	9-5	9-4	9-8	9-6	9-7	9-6
23	M	6-6	6-6	6-8	6-4	6-12	6-6	6-4	6-9	6-2	5-14	6-5	6-6
24	M	8-2	8-8	8-10	8-8	8-9	8-8	8-12	8-14	8-14	8-6	8-5	8-8
25	M	8-12	8-13	8-15	8-14	8-14	8-14	9-3	8-14	8-10	8-10	8-10	8-8
26	F	7-0	6-6	5-15	5-11	--	--	--	--	--	--	--	--
27	F	8-10	8-11	8-8	8-10	8-13	8-15	8-11	8-2	8-3	8-2	8-0	7-10
28	F	9-8	9-7	10-0	9-12	9-7	9-4	9-0	9-5	9-8	9-2	9-8	9-4
29	F	8-12	8-8	8-8	8-7	8-13	8-4	8-6	8-2	8-2	8-6	8-4	8-1

TABLE 20 (CONTINUED)

WEIGHT CHANGES IN POUNDS AND OUNCES DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	25	26	27	28	29	30	31	32	33	34	35	36	37
GROUP A														
1	M	10-8	9-13	10-6	9-11	9-6	8-14	9-2	9-1	9-5	9-2	9-7	9-0	9-6
2	M	10-6	10-8	10-3	10-8	10-8	10-6	10-0	10-4	10-2	9-10	9-8	9-8	9-7
5	F	6-6	6-5	6-4	6-4	5-15	6-8	6-4	6-6	6-2	6-1	6-7	6-10	6-2
6	F	6-7	5-13	6-8	6-11	6-10	6-11	6-10	6-10	6-10	6-9	7-2	6-14	6-12
7	F	8-12	8-8	8-8	7-12	8-0	8-0	7-14	8-2	8-5	8-6	8-6	8-6	8-6
8	F	5-6	5-6	5-9	5-6	5-6	5-6	5-6	5-6	5-7	5-10	5-10	5-8	5-10
GROUP B														
13	M	9-4	9-2	9-3	9-3	9-4	9-0	9-4	9-4	9-8	9-8	9-10	9-10	9-12
14	M	9-4	9-0	9-8	9-3	9-2	9-3	9-8	9-6	9-8	9-9	9-7	9-7	9-11
15	M	9-5	9-2	9-4	9-2	9-0	8-14	9-0	8-13	8-14	8-12	9-2	8-10	8-12
16	M	11-10	11-4	--	--	--	--	--	--	--	--	--	--	--
18	F	7-9	7-1	7-1	7-4	6-14	7-6	7-4	7-4	7-14	8-0	7-14	7-8	7-10
19	F	5-10	5-1	5-15	5-8	5-12	5-6	5-12	5-6	5-8	5-7	5-9	5-8	5-10
20	F	6-11	7-2	7-2	7-4	7-2	7-4	7-6	7-8	7-10	8-0	8-11	8-8	8-8
21	F	5-9	5-14	5-15	5-12	5-4	5-4	5-6	5-4	5-1	5-2	5-0	5-2	--
GROUP C														
22	M	9-8	9-8	9-15	9-2	8-13	8-13	8-14	8-12	9-0	8-14	9-8	9-0	9-0
23	M	6-6	6-0	6-10	6-12	6-1	6-4	6-0	6-0	6-6	6-1	6-7	5-14	6-5
24	M	8-11	8-8	8-8	8-9	8-10	8-8	8-14	8-12	8-13	8-10	8-12	8-14	8-12
25	M	8-9	8-4	8-7	8-4	8-4	8-2	8-4	8-6	8-4	8-7	8-9	8-10	8-8
26	F	--	--	--	--	--	--	--	--	--	--	--	--	--
27	F	7-0	7-2	6-2	5-15	--	--	--	--	--	--	--	--	--
28	F	9-2	9-1	9-0	9-2	9-2	9-1	8-14	8-12	8-14	9-0	9-4	9-4	9-4
29	F	8-0	8-2	8-5	8-2	8-0	8-0	7-14	8-0	8-0	7-14	7-12	7-10	7-8

TABLE 21

WEIGHT CHANGES DURING THE COURSE OF INFECTION

WEEK NO.	GROUP A		GROUP B		GROUP C	
	MALE	FEMALES	MALE	FEMALES	MALES	FEMALES
0	9.3 ± 1.1*	6.7 ± 1.2	8.6 ± 0.2	6.2 ± 1.0	7.4 ± 1.0	7.4 ± 1.2
1	9.5 ± 1.7	6.5 ± 1.2	8.7 ± 0.3	5.9 ± 1.1	7.5 ± 1.1	7.7 ± 1.0
1	9.8 ± 1.4	6.8 ± 0.9	8.8 ± 0.6	6.3 ± 1.3	8.0 ± 0.7	8.0 ± 1.1
2	9.9 ± 1.5	6.8 ± 1.0	9.8 ± 0.3	6.1 ± 1.1	7.8 ± 1.2	7.9 ± 1.3
3	10.0 ± 1.4	6.8 ± 1.1	9.1 ± 0.5	6.4 ± 0.9	7.8 ± 1.1	8.1 ± 1.3
5	10.2 ± 1.7	6.8 ± 0.9	9.2 ± 0.4	6.4 ± 1.2	8.3 ± 1.4	7.4 ± 1.7
6	10.4 ± 1.4	6.7 ± 0.8	9.3 ± 0.6	6.4 ± 1.3	8.3 ± 1.4	8.3 ± 1.3
7	10.5 ± 1.5	6.8 ± 0.9	9.3 ± 0.4	6.4 ± 1.4	8.0 ± 1.1	8.5 ± 1.3
8	10.8 ± 1.1	6.7 ± 0.9	9.4 ± 0.2	6.6 ± 1.5	7.9 ± 1.1	7.8 ± 1.0
9	10.6 ± 1.4	6.6 ± 0.9	9.2 ± 0.4	6.4 ± 1.2	7.9 ± 1.1	7.8 ± 1.0
10	10.6 ± 1.1	6.7 ± 1.2	9.2 ± 0.2	6.3 ± 0.9	7.6 ± 1.1	8.0 ± 1.5
11	10.6 ± 0.7	6.9 ± 1.0	9.5 ± 0.2	6.3 ± 0.9	7.9 ± 1.2	8.2 ± 1.4
12	10.5 ± 0.5	6.8 ± 1.1	9.4 ± 0.4	6.9 ± 0.9	8.0 ± 0.9	8.1 ± 1.5
13	10.6 ± 0.7	6.9 ± 1.2	9.5 ± 0.1	6.6 ± 1.1	8.1 ± 1.2	8.5 ± 1.1
14	10.5 ± 0.7	6.9 ± 1.2	9.7 ± 0.3	6.7 ± 1.1	8.2 ± 1.2	8.3 ± 1.3
15	10.8 ± 0.4	7.1 ± 1.2	10.1 ± 0.3	6.7 ± 1.3	8.4 ± 1.3	8.3 ± 1.7
16	10.7 ± 0.1	6.9 ± 1.1	10.0 ± 1.2	6.8 ± 1.2	8.4 ± 1.5	8.1 ± 1.7
17	10.6 ± 0.4	7.0 ± 1.3	9.9 ± 0.1	6.5 ± 1.2	8.4 ± 1.2	9.0 ± 0.4
18	10.5 ± 0.2	7.3 ± 0.9	9.8 ± 0.2	6.7 ± 1.1	8.3 ± 1.3	8.8 ± 0.5

*GROUP AVERAGE ± 1 STANDARD DEVIATION

TABLE 21 (CONTINUED)

WEIGHT CHANGES DURING THE COURSE OF INFECTION

WEEK NO.	GROUP A		GROUP B		GROUP C	
	MALE	FEMALES	MALE	FEMALES	MALES	FEMALES
19	10.3 ± 0.2	7.0 ± 1.5	9.6 ± 0.1	6.7 ± 1.3	8.3 ± 1.4	8.7 ± 0.3
20	10.8 ± 0.4	7.2 ± 1.4	9.7 ± 0.3	6.7 ± 1.4	8.5 ± 1.3	8.5 ± 0.7
21	10.9 ± 0.3	7.0 ± 1.7	9.6 ± 0.3	6.7 ± 0.3	8.3 ± 1.5	8.6 ± 0.8
22	11.3 ± 0.8	7.2 ± 1.6	9.5 ± 0.2	6.2 ± 0.9	8.1 ± 1.6	8.5 ± 0.5
23	10.8 ± 0.3	7.2 ± 1.7	10.1 ± 0.3	6.3 ± 1.8	8.2 ± 1.3	8.9 ± 0.8
24	10.5 ± 0.1	7.0 ± 1.6	9.4 ± 0.3	6.4 ± 1.1	8.2 ± 1.3	8.3 ± 0.9
25	10.4 ± 0.1	6.7 ± 1.4	9.3 ± 0.0	6.4 ± 1.0	8.3 ± 1.3	8.0 ± 1.1
26	10.2 ± 0.5	6.5 ± 1.4	9.1 ± 0.1	6.3 ± 1.0	8.0 ± 1.3	8.1 ± 1.0
27	10.3 ± 0.1	6.6 ± 1.0	9.3 ± 0.2	6.5 ± 0.2	8.4 ± 1.4	7.8 ± 1.5
28	10.1 ± 0.6	6.7 ± 1.3	9.2 ± 0.1	6.4 ± 0.9	8.2 ± 1.0	7.7 ± 1.6
29	10.0 ± 0.8	6.4 ± 1.0	9.1 ± 0.1	6.3 ± 0.9	7.9 ± 1.3	8.6 ± 0.8
30	9.7 ± 1.0	6.6 ± 1.1	9.0 ± 0.2	6.3 ± 1.2	7.9 ± 1.2	8.5 ± 0.7
31	9.6 ± 0.6	6.6 ± 1.1	9.3 ± 0.2	6.4 ± 1.0	8.0 ± 1.4	8.5 ± 0.7
32	9.7 ± 0.8	6.6 ± 1.0	9.2 ± 0.3	6.3 ± 1.2	8.0 ± 1.3	8.4 ± 0.5
33	9.7 ± 0.6	6.6 ± 1.1	9.3 ± 0.4	6.5 ± 1.4	8.1 ± 1.2	8.4 ± 0.6
34	9.4 ± 0.6	6.7 ± 1.2	9.3 ± 0.5	6.6 ± 1.6	8.0 ± 1.3	8.4 ± 0.8
35	9.3 ± 0.4	6.9 ± 1.1	9.4 ± 0.3	6.8 ± 1.8	8.3 ± 1.3	8.5 ± 1.1
36	9.3 ± 0.3	6.8 ± 1.2	9.2 ± 0.5	6.7 ± 1.6	8.1 ± 1.5	8.4 ± 1.1
37	9.4 ± 0.1	6.7 ± 1.2	9.4 ± 0.6	7.3 ± 1.5	8.1 ± 1.2	8.4 ± 1.2

APPENDIX B

TABLE 22

THE PERCENTAGE OF EOSINOPHILS PRESENT IN THE SERUM OF EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	1		0		4		5		8		3		9		3		1	
2	M	2	1		1		5		2		2		6		8		1		2
5	F	2	3		5		1		3		1		5		7		5		3
6	F	2	0		5		3		5		5		3		3		13		3
7	F	1		4		6		5		3		1		2		2		2	
8	F	1		0		5		10		9		7		4		6		2	
	AVE.	1	2	2	5	6	2	8	4	7	3	4	4	3	5	4	9	2	3
GROUP B																			
13	M	1		1		4		3		12		4		8		6		11	
14	M	2	3		6		6		5		8		4		5		6		7
15	M	1	3		2		1		0		5		5		4		4		5
16	M				9	5		2		2		5		5		10		5	
18	F	1	4		10		13		6		7		10		17		18		6
19	F	2	2		13		7		3		11		9		7		5		5
20	F	2		3		9		10		5		4		10		6		6	
21	F	2		2		10		3		18		4		11		7		7	
	AVE.	2	3	3	12	4	10	7	5	12	9	4	10	11	12	7	12	7	6
GROUP C																			
22	M	1	2		12		2		4		4		4		15		5		6
23	M	2		2		8		5		8		6		15		12		1	
24	M	1		2		10		13		14		12		8		6		6	
25	M	3	2		10		6		1		10		8		13		5		8
26	F	2		3		4		22		8		5		14					
27	F	3	4		8		18		3	7	6		11		5		26		12
28	F	2	0		10		28		8	6	14		23		19		18		6
29	F	5		2		12		12		18		6		13		8		3	
	AVE.	3	2	3	9	3	23	4	4	10	12	6	17	14	12	9	22	3	8

TABLE 22 (CONTINUED)

THE PERCENTAGE OF EOSINOPHILS PRESENT IN THE SERUM OF EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	2		4		4		2		3		4
2	M		8		3		2		3		3	
5	F		5		3		2		5		6	
6	F		4		7		3		6		3	
7	F	2		3		6		4		8		8
8	F	3		3		4		2		8		5
	AVE.	3	6	3	4	5	2	2	5	6	4	6
GROUP B												
13	M	7		5		11		10		8		
14	M		7		8		10		10		8	
15	M		7		6		7		3		8	
16	M	4		8		13		9		6		5
18	F		9		7		8		7		11	
19	F		19		11		13		7		9	
20	F	3		4		9		7				7
21	F	9		6		10		6				7
	AVE.	6	10	6	8	11	10	8	7	7	9	6
GROUP C												
22	M		8		10		10		3		13	
23	M	7		7		9		12		15		
24	M	7		9		10		8		8		8
25	M		9		10		11		5		3	
26	F											
27	F		7		12		10					
28	F		12		11		11		7		12	
29	F	6		12		8		12		16		7
	AVE.	7	9	9	11	9	11	10	5	13	9	8

TABLE 23

THE NUMBER OF SERUM EOSINOPHILS EXPRESSED AS NUMBER EOSINOPHILS/mm^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP	A														
1	M	140		0		484		520		944		465		1,197	
2	M	338	111		101		490		250		308		888		1,296
5	F	201	468		730		101		336		94		860		679
6	F	294	0		710		483		520		660		465		273
7	F	745		672		594		460		342		98		198	
8	F	37		0		605		1,050		774		595		504	
	AVE.	181	198	224	514	561	358	677	369	687	531	406	738	633	747
GROUP	B														
13	M	150		197		452		633		1,212		592		1,248	
14	M	375	272		540		786		615		888		404		830
15	M	135	261		316		118		-		450		610		496
16	M				1,540	940		334		256		710		575	
18	F	145	584		1,534		1,534		912		714		1,140		2,074
19	F	206	268		1,196		560		312		1,166		1,431		917
20	F	172		363		1,314		1.180		805		448		1,040	
21	F	466		284		1,880		675		3,186		512		1,782	
	AVE.	236	346	281	1,026	1,149	750	706	616	1,365	805	566	896	1,161	1,080
GROUP	C														
22	M	134	334		1,968		228		472		644		644		2,310
23	M	290		310		704		720		784		528		1,815	
24	M	187		196		1,640		2,440		1,792		1,692		896	
25	M	471	402		1,120		744		138		1,180		1,304		1,820
26	F	512		564		368		3,520		1,584		810		2,450	
27	F	336	544		1,400		3,708		318	1,120	546		1,475		490
28	F	173			1,140		4,956		1,448	864	1.288		4,255		2,812
29	F	815		294		1,944		1,210		1,440		792		2,353	
	AVE.	365	426	341	1,452	1,164	2,700	2,648	594	1,368	915	956	1,925	1,879	1,858

TABLE 23 (CONTINUED)

THE NUMBER OF SERUM EOSINOPHILS EXPRESSED AS NUMBER EOSINOPHILS/mm^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	16	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP	A															
1	M	456		250		240		432		632		234		375		488
2	M		136		400		1,000		303		288		450		306	
5	F		500		264		610		294		292		505		696	
6	F		1,833		390		632		994		603		1,128		534	
7	F	176		222		178		366		636		496		1,100		1,024
8	F	534		196		426		345		456		228				550
	AVE.	389	823	223	351	281	747	381	530	575	394	319	694	738	512	687
GROUP	B															
13	M	756	744	2,090	784	1,288	1,022	715	1,184	1,331	1,200	1,240	1,450		1,128	
14	M		744		784		1,022		1,184		1,200		1,450		1,128	
15	M		632		710		1,057		828		854		384		1,072	
16	M	1,160		885		620		1,176		2,015		1,044		846		620
18	F		3,168		852		1,395		966		1,504		777		1,705	
19	F		720		600		4,050		1,012		1,833		644		882	
20	F	828		800		372		580		1,188		896				931
21	F	1,855		2,940		1,728		828		1,829		793				868
	AVE.	1,150	1,316	1,679	737	1,002	1,881	825	998	1,340	1,350	998	814	999	1,197	806
GROUP	C															
22	M		855		1,092		1,280		1,620		1,890		423		1,339	
23	M	1,620		95		623		1,085		990		1,428		1,920		
24	M	672		948		1,127		1,458		950	1,048	1,048		912		928
25	M		560		1,120		1,458		1,040		1,694		700		267	
26	F		2,755		690		1,480		1,246		1,029					
27	F		3,380		720		1,099		1,128		980					
28	F		2,129		660		1,860		1,364		1,078		777		1,512	
29	F	1,504		597		1,086		1,896		1,040		1,500		2,800		1,085
	AVE.	1,265	1,730	567	898	945	1,424	1,480	1,288	993	1,411	1,328	633	1,877	1,039	1,007

TABLE 24

SERUM GLUTAMIC PYRUVIC TRANSAMINASE VALUES EXPRESSED AS SF UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	29		30		34		25		26		36		30		24		24	
2	M	25	28		36		20		42		26		24		26		28		22
5	F	35	32		35		32		24		22		32				40		24
6	F	32	30		40		20		26		28		20		30		22		22
7	F	26		32		30		23		22		28		30		26		22	
8	F	48		48		31		39		32		40		30		30		24	
	AVE.	33	30	37	37	34	24	29	31	27	25	35	25	30	28	27	30	23	23
GROUP B																			
13	M	29		26		28		26		28		26		48		24		26	
14	M	34	22		30		21		68		40		44		44		24		30
15	M	35	30		30		24		20		37		30		44		26		18
16	M				45	43		50		36		30		26		30		32	
18	F	33	30		35		38		28		42		38		42		35		24
19	F	39	38		42		38		48		22		32		30		26		32
20	F	46		40		48		40		32		44		48		54		42	
21	F	46		20		32		40		37		43		40		37		38	
	AVE.	37	30	29	36	38	30	39	41	34	35	36	34	41	40	36	28	35	26
GROUP C																			
22	M	29	28		28		30		46		92		50		60		54		44
23	M	27		28		32		35		26		36		48		22		28	
24	M	33		48		34		41		107		26		32		37		22	
25	M	32	24		40		27		26		44		42		38		22		22
26	F	46		72		42		84		68		101		88		-		-	
27	F	35	28		52		38		26	22	34		30		42		32		22
28	F	27	30		51		64		30	28	72		132		60		80		42
29	F	38		30		130		102		72		88		72		58		48	
	AVE.	33	28	45	43	60	40	66	32	54	61	63	64	60	50	39	47	33	35

TABLE 24 (CONTINUED)

SERUM GLUTAMIC PYRUVIC TRANSAMINASE VALUES EXPRESSED AS SF UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	20		28		24		36		22		24
2	M		28		24		26		24		26	
5	F		28		24		38		24		28	
6	F		24		24		30		26		26	
7	F	22		28		24		26				22
8	F	30		24		26		32		28		22
	AVE.	24	27	27	24	25	31	31	25	25	26	22
GROUP B												
13	M	26		24		22		26				
14	M		30		30		28		30		36	
15	M		28		24		28		22		28	
16	M	36		26		32		40		30		26
18	F		26		24		32		26		26	
19	F		28		30		28		28		28	
20	F	48		30		32		36				42
21	F	32		32		30		32				30
	AVE.	36	28	28	27	29	29	34	27	30	30	33
GROUP C												
22	M		30		38		32		38		32	
23	M	30		44		28		30				
24	M	40		26		28		32		28		22
25	M		28		36		48		42		30	
26	F	-										
27	F		30		22		26					
28	F		35		32		40		32		48	
29	F	84		42		32						30
	AVE.	51	31	37	32	30	37	34	37	28	37	26

TABLE 25

SERUM GLUTAMIC OXALOACETIC ACID TRANSAMINASE VALUES EXPRESSED AS SF UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	33		28		54		22		29		34		32		27		46	
2	M	32	32		32		28		35		38		46		26		32		38
5	F	32	44		26		38		22		42		52		–		46		26
6	F	41	38		64		38		26		25		38		50		28		30
7	F	26		32		52		28		20		24		34		29		28	
8	F	46		38		40		12		26		60		48		44		36	
	AVE.	35	38	33	41	49	35	13	28	25	35	39	45	38	38	33	35	37	31
GROUP B																			
13	M	28		30		38		30		40		32		50		27		40	
14	M	33	32		30		30		92		52		52		32		27		28
15	M	37	34		32		28		24		25		44		38		30		26
16	M				40	38		46		20		46		46		34		50	
18	F	33	40		33		38		38		34		38		32		25		34
19	F	35	40		38		30		52		44		32		36		30		44
20	F	39		32		67		40		18		34		56		61		46	
21	F	50		20		40		26		22		32		50		42		50	
	AVE.	36	37	27	35	46	32	36	52	25	39	36	34	51	35	41	28	47	33
GROUP C																			
22	M	29	28		28		30		46		92		50		60		62		38
23	M	33		30		36		39		28		24		70		25		36	
24	M	29		38		68		44		72		34		34		42		28	
25	M	34	32		43		28		28		52		50		53		25		38
26	F	33		32		31		50		56		52		96					
27	F	42	50		60		28		38	16	46		60		53		37		38
28	F	28	43		44		40		30	32	46		108		44		50		38
29	F	43		32		106		74		54		94		108		66		56	
	AVE.	34	36	33	44	60	32	52	36	43	59	51	67	77	53	44	44	40	38

TABLE 25 (CONTINUED)

SERUM GLUTAMIC OXALOACETIC ACID TRANSAMINASE VALUES EXPRESSED AS SF UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

		WEEKS POST-INFECTION										
CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	20		30		30		44		24		26
2	M		22		26		35		30		34	
5	F		30		40		40		38		28	
6	F		28		26		40		30		30	
7	F	28		30		28		30				26
8	F	24		28		34		34		38		30
	AVE.	26	27	30	31	31	38	36	33	31	31	27
GROUP B												
13	M	34		30		32		32				
14	M		22		28		35		38		28	
15	M		26		30		22		28		28	
16	M	28		38		34		50		30		30
18	F		28		28		26		28		24	
19	F		28		44		38		28		28	
20	F	38		32		34		30				32
21	F	44		30		46		38				26
	AVE.	36	26	33	33	37	30	38	31	30	27	29
GROUP C												
22	M		24		30		44		30		30	
23	M	28		46		46		44				
24	M	24		28		38		32		36		28
25	M		30		50		38		38		32	
26	F											
27	F		34		26		38					
28	F		32		34		30		30		44	
29	F	52		46		42		50				35
	AVE.	35	30	40	35	42	38	42	33	36	35	32

TABLE 26

SERUM TOTAL BILIRUBIN EXPRESSED AS mg/100 ml FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17
Group A																		
1	M	0.3		0.2		0.0		0.2		-		0.0					0.2	
2	M	0.2	0.1		0.1		0.2		0.2		0.2		0.2		0.1			0.2
5	F	0.3	0.4		0.3		0.5		0.2		0.2		0.0		0.0			0.1
6	F	0.2	0.2		0.3		0.2		0.2		0.3		0.3		0.1			0.1
7	F	0.2		0.3		0.2		0.3		0.2		-		0.3			0.3	
8	F	0.2		0.2		0.2		-		0.2		0.2		0.2			0.2	
	AVE.	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1	0.2	0.3	0.1		0.2	0.1
Group B																		
13	M	0.3		-		0.2		0.4		-		0.3		0.5			0.2	
14	M	0.3	0.2		0.2		0.2		0.4		0.4		0.2		0.2			0.2
15	M	0.2	0.2		0.2		0.2		0.2		0.2		0.3		0.3			0.2
16	M				0.2	0.2		0.2		-		0.2		0.2		-	0.2	
18	F	0.2	0.2		0.2		0.2		0.2		0.2		0.2		0.4			0.3
19	F	0.2	0.3		0.2		0.2		0.2		0.3		0.4		0.5		0.3	
20	F	0.2		-		0.2		0.3		-		0.4		0.4			0.1	
	AVE.	0.2	0.2	-	0.2	0.2	0.2	0.3	0.2	-	0.3	0.3	0.3	0.4	0.4		0.2	0.3
Group C																		
22	M	0.3	0.2		0.1		0.2		0.4		1.8		0.3		0.4			0.2
23	M	0.4		0.3		0.3		1.5		-		2.8		3.4			1.4	
24	M	0.2		0.2		0.2		0.4		-		0.2		0.2			0.2	
25	M	0.2			0.3		0.2		0.2		0.2		0.2		-			0.3
26	F	0.2		0.5		0.6		0.5		-		0.5		2.8		4.0	3.7	
27	F	0.2	0.2		0.3		0.2		0.2		0.2				0.3			0.2
28	F	0.3	-		-		-		0.4	0.6	0.8		0.4		-			0.3
29	F	0.3		0.2		0.2		0.2		-		0.3		0.3			0.3	
	AVE.	0.3	0.2	0.3	0.3	0.3	0.2	0.7	0.3	0.6	0.8	1.0	0.3	1.7	0.4	4.0	1.4	0.3

TABLE 26 (CONTINUED)

SERUM TOTAL BILIRUBIN EXPRESSED AS mg/100 ml FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	18	19	20	21	22	23	24	25	27	30	31	35	38
Group A														
1	M	0.2		0.2		-		0.2		0.2				0.2
2	M		-		0.1		0.1		0.1		0.2		0.2	
5	F		0.2		0.2		0.4		0.2		0.2		0.2	
6	F		0.2		0.2		0.2		-		0.2		0.2	
7	F	0.2		0.2		0.2		0.2						0.2
8	F	0.1		0.2		0.1		0.2						0.2
	AVE.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	0.2	0.2
Group B														
13	M	0.2		0.2		0.2		0.0						1.2
14	M		0.2		0.2		0.2		0.2		-		-	
15	M		0.2		0.2		0.2		0.2		0.2		0.2	
16	M	0.1		0.2		0.1		0.2						0.2
18	F		0.2		0.2		0.3		-		0.2		0.2	
19	F		-		0.2		0.2		0.2		0.2		0.2	
20	F	0.3		0.2		0.2		0.2						0.2
21	F	-		0.2		-		0.2						0.2
	AVE.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		0.2	-	0.2	0
Group C														
22	M		0.2		-		0.2		0.2		-		-	
23	M	-		0.6		-		0.8		0.2				-
24	M	0.2		0.4		0.2		0.2		-				0.3
25	M		0.2		-		0.2		0.2		0.4			0.3
26	F													
27	F		0.2		0.2		0.2		-					
28	F		0.2		-		0.2		0.2		0.3		0.2	
29	F	0.2	0.2	0.2		0.2		0.2		-				0.2
	AVE.	0.2	0.2	0.4	0.2	0.2	0.2	0.4	0.2	0.2	0.4	-	0.2	0.3

TABLE 27

SERUM ALKALINE PHOSPHATASE ENZYME VALUES EXPRESSED IN BODANSKI UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17
GROUP A																	
1	M	5.0		2.2		3.0		3.0		2.6		2.5		2.0		2.2	
2	M	1.2	1.1		0.9		1.4		0.8		1.5		1.9		1.0		1.2
5	F	5.0	4.0		1.8		2.5		3.0		1.5		1.8				1.4
6	F	6.0	2.0		4.7		1.5		1.8		2.5		1.8		2.5		2.8
7	F			2.2		2.3		2.3		1.8		1.8		2.2		2.2	
8	F			2.2		2.0		1.5		2.5		2.0		1.6		1.5	
	AVE.		2.4	2.2	2.5	2.4	1.8	2.3	1.9	2.3	1.8	2.1	1.8	1.9	1.8	2.0	1.8
GROUP B																	
13	M			2.0		4.0		2.3		1.5		2.2		1.6		1.5	
14	M	5.6	2.0		1.8		2.0		3.0		3.0		1.8		3.0		1.2
15	M		1.5		2.0		1.2		1.8		2.2		1.6		2.4		1.4
16	M				3.0	3.0		3.0		1.8		2.4		1.6		3.0	
18	F	5.0	3.6		4.4		5.2		5.0		4.5		4.0		4.5		1.2
19	F		2.5		1.8		1.2		3.0		1.4		1.8		2.0		1.6
20	F			2.6		3.5		2.0		1.6		3.0		4.0		2.0	
21	F			2.2		3.0				1.5		2.2		1.6		1.8	
	AVE.		2.4	2.3	2.6	3.4	2.4	2.4	3.2	1.6	2.8	2.5	2.3	2.2	3.0	2.1	1.4
GROUP C																	
22	M	4.0	2.5		2.8		2.2		1.4		6.5		2.2		3.5		2.5
23	M			3.2		2.3		1.3		1.3		1.2		2.5		1.8	
24	M			3.0		2.5		2.8		1.3				1.4		1.5	
25	M		2.5		3.0		3.0		1.8		1.6		1.8		2.5		1.4
26	F			5.0		1.8		2.5		2.5		3.0		3.0			
27	F	5.0	1.0		3.0		1.4		1.4	1.0	2.2		1.8		3.2		1.8
28	F	3.8	2.5		1.5		1.4		1.8	1.8	2.2		3.5		3.		3.8
29	F			2.0		6.0		4.0		2.5		3.8		5.8		3.0	
	AVE.		2.1	3.3	2.6	3.2	2.0	2.6	1.6	1.3	3.1	2.3	2.3	3.2	3.2	2.1	2.4

TABLE 27 (CONTINUED)

SERUM ALKALINE PHOSPHATASE ENZYME VALUES EXPRESSED IN BODANSKI UNITS FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

		WEEKS POST-INFECTION												
CAT #	SEX	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A														
1	M	2.9		1.5		1.5		1.0		1.8				1.0
2	M		1.5		1.5				1.8		1.2		2.0	
3	F		1.0		1.5		2.5		1.5		1.5		1.5	
4	F		2.0		1.6		3.0		1.5		2.2		2.0	
7	F	1.5		2.5		1.5		1.5		1.3				1.5
8	F	1.5		3.0		1.0		1.4		1.8				1.8
	AVE.	2.0	1.5	2.3	1.5	1.5	2.7	1.3	1.6	1.6	1.6		1.8	1.4
GROUP B														
13	M	2.1		1.5		1.0		1.8		1.8				
14	M		2.2		1.4		2.5		1.8		1.2		2.0	
15	M		1.5		1.4		2.5		1.5		1.2		1.5	
16	M	1.5		1.8		1.0		1.5		1.3				2.5
18	F		1.5		1.2		2.5		1.8		1.5		1.5	
19	F		3.0		1.2		4.8		1.5		1.8		1.5	
20	F	2.2		3.4		1.5		1.8		1.3				1.5
21	F	6.5		1.5		1.0		1.8		1.8				1.2
	AVE.	3.1	2.1	2.1	1.3	1.1	3.1	1.7	1.7	1.6	1.4		1.6	1.7
GROUP C														
22	M		1.8		2.0		3.2		2.0		2.2		2.2	
23	M	2.1		1.5		2.2		1.5		1.3				
24	M	1.5		2.8		1.0		1.8		1.3				1.5
25	M		1.5		2.2		4.0		1.5		1.8		2.2	
26	F													
27	F		1.5		1.4		3.0		1.5					
28	F		1.5		1.5		3.0		1.8		1.5		2.0	
29	F	2.5		4.0		2.5		2.0		2.1				1.5
	AVE.	2.0	1.6	2.8	1.8	1.9	3.3	1.8	1.7	1.6	1.8		2.1	1.5

TABLE 28

ICTERUS INDEX VALUES FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	3		3		3		3		3		3		3		3		3	3
2	M	3	3		3		3		3		3		3		3		3		3
5	F	3	3		3		3		5		3		3		3		3		3
6	F	3	3		3		3		3		3		3		3		5		3
7	F	3		3		3		3		3		3		3		3		3	
8	F	3		5		3		3		3		5		3		3		3	
GROUP B																			
13	M	3		3		3		5		3		3		3		3		3	
14	M	3	3		3		3		3		5		5		3		3		-
15	M	3	3		3		3		3		5		5		5		3		3
16	M				5	3		3		3		3		3		4		3	
18	F	3	3		3		3		3		3		3		5		3		-
19	F	3	3		3		3		3		3		3		3		3		5
20	F	3		3		3		3		3		5		5		3		3	
21	F																		
GROUP C																			
22	M	3	3		3		3		3		10		6		3		3		3
23	M	4		3		3		3		5		3		5		3		3	
24	M	3		3		3		3		3		3		3		3		3	
25	M	3	3		5		5		3		3		3		3		3		3
26	F	3		5		3		3		5		10		10					
27	F	4	3		3		3		3	7	3		5		5		3		3
28	F	4	5		3		3		3	3	5		6		9		5		3
29	F	3		3		3		3		3		3		3		3		3	

TABLE 28 (CONTINUED)

ICTERUS INDEX VALUES FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M			5		3		3		3		3
2	M		3		3		3		3		3	
5	F		3		3		3		3		3	
6	F		3		3		3		3		3	
7	F	3		3		3		3				3
8	F	3		3		3		3		3		5
GROUP B												
13	M	3		3		3		3		3		
14	M		3		3		4		3		3	
15	M		5		3		3		3		3	
16	M	3		3		3		3		3		3
18	F		3		3		3		3		3	
19	F		3		3		3		3		3	
20	F	3		3		3		3				3
21	F			5		3		3				3
GROUP C												
22	M		7		3		3		3		3	
23	M	3		3		3		5		3		
24	M	3		3		3		3		3		3
25	M		3		5		3		3		3	
26	F											
27	F		3		3		3					
28	F		3		3		3		3		3	
29	F	3		3		3						3

TABLE 29

HEMATOCRIT VALUES EXPRESSED AS PERCENT FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	38		35		45		46		38		42		44		40		40	
2	M	36	34		35		35		35		45		36		38		34		26
5	F	44	45		40		34		44		46		48		36		42		44
6	F	36	42		33		40		30		40		40		40		36		30
7	F	36		38		35		42		30		40		46		36		30	
8	F	41		40		36		44		36		40		36		37		35	
	AVE.	39	40	38	36	39	36	44	36	35	44	41	41	42	38	38	37	33	37
GROUP B																			
13	M	40		48		48		50		41		44		35		40		45	
14	M	42	48		52		50		45		45		44		50		48		50
15	M	43	35		41		42		40		35		30		35		36		35
16	M				52	46		46		50		45		40		54		50	
18	F	44	42		45		42		44		40		42		50		40		45
19	F	40	44		40		35		38		38		40		42		36		32
20	F	43		35		50		45		35		42		48		42		48	
21	F	40		50		40		34		44		45		48		50		45	
	AVE.	42	42	46	47	46	42	45	42	43	40	44	39	43	44	47	40	47	41
GROUP C																			
22	M	38	42		49		48		40		45		45		45		35		45
23	M	48		42		45		32		41		40		40		38		35	
24	M	46		44		50		56		46		32		32		34		35	
25	M	45	45		39		35		40		40		34		32		33		38
26	F	38		38		30		45		36		40		25		-		-	
27	F	37	40		42		36		32	24	34		30		35		35		35
28	F	39	35		40		40		34	33	34		33		40		40		35
29	F	37		42		42		38		39		40		42		42		25	
	AVE.	41	41	42	43	42	40	43	37	37	38	38	36	35	38	38	36	32	38

TABLE 29 (CONTINUED)

HEMATOCRIT VALUES EXPRESSED AS PERCENT FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	40		38		41		48		40		50
2	M		45		35		40		45		45	
5	F		40		44		45		40		40	
6	F		23		32		30		35		35	
7	F	26		38		34		32				45
8	F	42		40		50		40		30		38
	AVE.	36	36	39	37	42	38	43	40	35	40	44
GROUP B												
13	M	36		42		50		42		36		
14	M		24		34		48		45		48	
15	M		38		40		42		35		40	
16	M	50		46		54		48		50		44
18	F		40		34		30		36		35	
19	F		28		40		40		40		35	
20	F	35		32		45		44				46
21	F	48		40		50		46				46
	AVE.	42	33	40	37	50	40	45	39	43	40	45
GROUP C												
22	M		45		45		40		52		45	
23	M	30		40		52		40		40		
24	M	40		36		50		50		48		48
25	M		30		44		40		44		40	
26	F	-										
27	F		35		35		40					
28	F		32		40		35		42		40	
29	F	36		44		38		40		32		40
	AVE.	36	36	40	41	47	38	46	46	40	42	44

TABLE 30

TOTAL ERYTHROCYTE COUNT EXPRESSED AS NO. RBC/mm^3 X 10^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16
GROUP A																
1	M	7.5		7.6		9.0		10.0		7.5		8.6		10.0		10.1
2	M	8.5	8.5		8.0		9.0		8.6		11.4		8.0		8.0	
5	F	8.9	9.6		7.6		7.5		9.6		10.1		10.1		8.5	
6	F	7.8	7.5		7.4		7.4		8.0		8.6		9.5		8.5	
7	F	8.0		7.5		9.0		8.5		6.8		8.0		10.1		8.5
8	F	10.0		9.5		9.6		10.0		8.0		9.8		8.6		11.2
	AVE.	8.5	8.5	8.2	7.7	9.2	8.0	9.5	8.7	7.4	10.0	8.8	9.2	9.6	8.3	9.9
GROUP B																
13	M	8.1		11.1		9.8		10.5		6.3		9.6		8.6		7.4
14	M	8.3	8.5		9.5		10.4		10.8		10.5		9.8		10.2	
15	M	9.3	7.8		8.1		9.2		9.5		8.5		6.4		10.1	
16	M				9.8	9.9		10.5		10.0		10.4		9.7		11.0
18	F	9.2	9.7		9.6		9.9		9.6		9.9		9.5		9.0	
19	F	9.2	10.0		9.7		8.0		8.2		9.9		9.6		9.5	
20	F	9.9		8.9		10.8		8.9		8.5		9.7		10.5		9.8
21	F	9.7		9.2		10.2		7.5		8.8		10.6		10.6		10.5
	AVE.	9.1	9.0	9.7	9.3	10.2	9.4	9.4	9.5	8.4	9.7	10.1	8.8	9.9	9.7	9.4
GROUP C																
22	M	7.8	10.0		10.2		10.2		10.0		10.0		10.0		8.2	
23	M	9.2		10.0		10.0		10.4		9.0		10.1		9.7		8.5
24	M	9.0		10.0		10.2		10.6		9.2		8.0		7.6		10.0
25	M	8.7	9.9		8.6		6.0		8.6		9.6		8.0		10.0	
26	F	7.9		9.5		7.7		9.3		7.4		8.5		6.0		
27	F	7.5	8.8		9.7		8.2		7.8	5.5	8.4		6.2		8.4	
28	F	8.0	8.0		9.4		9.7		8.9	6.4	8.6		7.6		8.4	
29	F	7.9		10.5		9.4		10.0		7.4		9.2		9.8		9.0
	AVE.	8.1	9.2	10.0	9.5	9.3	8.5	10.0	8.8	7.5	9.2	9.0	8.0	8.3	8.8	9.2

TABLE 30 (CONTINUED)

TOTAL ERYTHROCYTE COUNT EXPRESSED AS NO. RBC/mm^3 X 10^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A															
1	M		5.8		6.5		7.4		8.0		10.2		9.0		10.2
2	M	8.6		7.6		8.8		8.4		9.6		10.2		10.2	
5	F	9.9		9.4		7.8		8.5		10.3		8.5		8.1	
6	F	9.0		6.3		5.8		7.0		9.7		10.0		8.0	
7	F		7.8		6.5		7.5		8.3		7.6		6.2		9.0
8	F		6.5		9.5		7.6		9.9		9.0				7.7
	AVE.	9.2	6.9	7.9	7.5	7.5	7.5	8.0	8.7	9.9	8.9	9.6	7.6	8.8	9.0
GROUP B															
13	M		8.8		9.0		8.0		9.9		9.3		8.4		
14	M	11.0		10.1		5.8		8.5		10.0		10.0		9.8	
15	M	10.0		8.8		8.0		9.2		10.1		9.4		9.0	
16	M		10.7		10.2		8.5		10.2		10.3		10.2		8.8
18	F	10.1		9.1		7.6		8.8		9.5		8.5		8.1	
19	F	10.2		7.3		6.2		8.5		9.5		10.2		8.5	
20	F		10.2		9.0		6.4		10.1		9.5				9.1
21	F		9.3		10.0		7.5		10.0		9.9				9.5
	AVE.	10.3	9.8	8.6	9.6	6.9	7.6	8.8	10.1	9.8	9.8	9.5	9.3	8.9	9.1
GROUP C															
22	M	9.9		10.0		8.8		10.2		9.9		10.0		8.9	
23	M		6.5		6.6		7.6		10.1		9.2		9.5		
24	M		9.9		9.3		7.4		9.8		10.0		10.1		9.8
25	M	8.5		8.5		6.2		10.1		9.7		10.0		8.5	
26	F														
27	F	8.3		7.0		7.0		8.7		9.7					
28	F	8.6		7.5		6.2		9.8		8.2		8.7		8.6	
29	F		7.6		9.0		8.2		9.0		9.0		7.6		8.0
	AVE.	8.8	8.0	8.3	8.3	7.1	7.7	9.7	9.6	9.4	9.4	9.6	9.1	8.7	8.9

TABLE 31

BLOOD HEMOGLOBIN CONTENT EXPRESSES AS GMS/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP A															
1	M	12.5		12.0		15.5		15.5		-		13.5		14.0	
2	M	12.3	11.0		12.5		12.5		12.4		14.5		11.0		11.4
5	F	14.7	14.7		14.5		12.0		14.8		15.0		14.8		13.5
6	F	12.2	9.5		12.0		11.0		11.5		12.4		12.4		13.5
7	F	11.7		12.5		13.5	13.5	13.0		-		12.3		14.6	
8	F	11.0		13.5		12.0		15.0		-		13.3		11.2	
	AVE.	12.4	11.5	12.5	12.9	14.1	12.0	14.8	12.8	-	14.1	13.2	12.3	13.5	12.5
GROUP B															
13	M	14.4		14.0		15.5		14.7				12.5		11.0	
14	M	13.3	15.0		17.0		15.5		14.8		15.4		13.6		15.0
15	M	14.7	12.5		13.7		14.0		13.0		12.5		9.0		14.0
16	M				16.0	15.5		15.0		-		15.0		12.4	
18	F	15.0	14.0		16.0		13.5		13.5		14.0		12.6		15.5
19	F	13.3	13.0		14.0		12.5		13.3		13.0		12.4		13.8
20	F	13.8		12.0		15.5		14.0		-		13.8		15.4	
21	F	12.3		15.0		14.0		11.3		-		14.3		15.8	
	AVE.	13.8	13.6	13.8	15.3	15.1	13.9	13.8	13.7	-	13.7	13.9	12.0	13.7	14.6
GROUP C															
22	M	12.6	13.5		16.5		16.5		13.5		15.4		13.8		14.5
23	M	15.8		14.0		15.0		12.3		-		12.3		12.4	
24	M	14.8		15.0		16.5		16.8		-		10.5		9.8	
25	M	14.8	13.0		13.0		12.5		13.8		12.5		10.4		10.5
26	F	10.8		12.5		10.5		12.0		-		13.3		7.4	
27	F	11.3	12.0		14.0		12.5		11.2		11.5		9.4		12.4
	AVE.	13.3	12.8	13.9	14.0	14.0	13.9	13.5	12.5	-	12.7	12.3	11.0	10.7	12.9

TABLE 31 (CONTINUED)

BLOOD HEMOGLOBIN CONTENT EXPRESSED AS GM/100 cc FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	16	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A																
1	M	15.0		13.5		13.0		12.2		13.5		15.0		12.4		15.4
2	M		12.0		9.8		13.6		10.5		13.5		16.5		14.4	
5	F		14.5		14.5		12.2		12.6		15.6		14.3		12.4	
6	F		12.5		10.0		7.6		12.0		11.5		11.5		11.0	
7	F	12.5		12.0		8.5		12.2		11.5		10.0		9.4		13.8
8	F	13.0		12.5		15.0		12.4		12.3		12.0				
	AVE.	12.9	12.8	12.9	11.0	12.4	11.8	12.3	11.4	12.7	13.5	13.3	14.1	10.9	12.6	13.8
GROUP B																
13	M	12.0		14.0		13.0		12.8		13.5				11.2		
14	M		17.0		17.5		7.4		10.0		14.8	12.6	13.0		15.0	
15	M		12.0		13.5		12.0		13.5		14.0		13.0		12.4	
16	M	16.5		16.0		16.5		15.0		15.5		15.8		15.4		13.4
18	F		13.5		15.5		12.4		9.5		11.0		12.3		11.0	
19	F		11.5		11.0		9.0		14.4		12.0		13.5		11.2	
20	F	13.5		16.5		13.0		10.2		14.5		13.4				15.0
21	F	15.0		15.0		16.0		12.4		15.0		14.5				14.8
	AVE.	14.3	13.5	15.4	14.4	14.6	10.2	12.6	11.9	14.6	13.0	14.1	13.2	13.3	12.4	14.4
GROUP C																
22	M		12.0		17.0		13.6		17.4		14.5		17.0		14.4	
23	M	14.0		12.5		11.0		12.2		12.0		12.1		13.0		
24	M	15.0		12.4		14.5		12.0		13.5		15.4		17.0		15.0
25	M		11.0		12.5		10.2		13.0		13.5		13.5		12.2	
26	F															
27	F		12.0		12.5		10.7		9.0		12.0					
28	F		13.5		12.5		10.0		13.5		13.0		14.0		13.5	
29	F	12.0		12.5		14.0		13.5		12.4		12.1		10.2		12.4
	AVE.	13.3	12.2	12.5	13.6	13.4	11.1	12.8	13.2	12.6	13.3	13.2	14.8	13.4	13.0	13.7

TABLE 32

SEDIMENTATION RATE VALUES EXPRESSED AS mm/HR, FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	0				6		7		8		8		3		5		8	
2	M	6	7		0		5		2		5		5		6		14		-6
5	F	8	7		5		7		7		13		4		2		9		4
6	F	19	36		24		30		36		17		10		11		14		13
7	F	9		15		8		16		21		14		3		7		7	
8	F	12		12		0		16		6		4		4		3		3	
	AVE.	9	17	14	10	5	14	13	15	12	12	9	6	3	6	5	12	4	7
GROUP B																			
13	M	3		6		6		15		20		4		4		-8		4	
14	M	1	10		7		9		3		7		4		9		9		5
15	M	7	-3		4		6		-1		-11		6		-2		4		3
16	M				9	6		8		1		6		3		5		4	
18	F	14	12		13		15		8		11		10		8		5		5
19	F	5	-		9		3		2		1		3		6		9		-2
20	F	8		12		8		10		12		5		4		2		6	
21	F	10		10		8		2		7		10		4		5		6	
	AVE.	7	6	9	8	7	8	9	4	10	2	6	6	4	5	1	7	5	3
GROUP C																			
22	M	0	1		4		6		3		7		3		9		10		10
23	M	9		4		2		19		13		4		3		5		-5	
24	M	13		8		9		6		14		9		6		1		3	
25	M	3	6		1		6		1		2		7		0		4		2
26	F	6		11		10		12		6		7		10					
27	F	2	3		9		2		-2	8	12		8		4		5		4
28	F	0	2		3		5		7	12	10		6		8		8		8
29	F	5		4		13		14		14		5		4		-1		17	
	AVE.	5	3	7	4	9	5	13	2	11	8	6	6	6	5	2	7	5	6

TABLE 32 (CONTINUED)

SEDIMENTATION RATE VALUES EXPRESSED AS mm/HR. FOR
EXPERIMENTALLY INFECTED DATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	5		4		5		4		10		1
2	M		3		-1		-1		5		5	
5	F		3		10		5		11		5	
6	F		6		4		7				8	
7	F	6		6		1		20		20		6
8	F	4		3		20		10				12
	AVE.	5	4	4	4	9	4	11	8	15	6	6
GROUP B												
13	M	-2		4		12		10		14		
14	M		8		6		5		9		4	
15	M		6		3		0		8		5	
16	M	0		7		12		3		0		8
18	F		3		6		-8		12		15	
19	F		10		2		6		12		5	
20	F	4		4		4		5				7
21	F	2		3		12		5				8
	AVE.	1	7	5	4	10	1		10	7	7	8
GROUP C												
22	M		3		4		-		12		10	
23	M	4		3		15		2		10		
24	M	3		4		18		8		3		3
25	M		6		5				10		5	
26	F											
27	F		4		7		-					
28	F		4		-1		-		13		4	
29	F	2		4		1		10		20		10
	AVE.	3	4	4	4	11	-	6	12	11	6	7

TABLE 33

MEAN CELL VOLUME EXPRESSED AS CUBIC MICRONS FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP	A														
1	M	51.1		46.6		50.3		46.0		50.7		48.8		44.0	
2	M	42.0	40.0		43.8		38.9		40.7		39.4		45.0		47.5
5	M	50.8	46.9		52.6		45.3		45.8		41.8		48.0		42.4
6	F	46.1	56.0		44.6		54.1		37.5		46.5		42.1		47.1
7	F	43.9		50.7		38.9		48.4		42.0		50.0		45.5	
8	F	37.4		42.1		37.5		44.0		45.0		40.8		41.9	
	AVE.	45.2	47.6	46.6	47.0	42.2	46.1	46.1	41.3	46.1	42.6	46.5	45.0	43.8	46.9
GROUP	B														
13	M	48.7		43.2		44.7		47.6		65.6		45.8		40.7	
14	M	49.4	56.5		54.7		48.1		41.7		42.9		44.9		49.0
15	M	46.6	44.9		50.6		45.7		42.1		41.2		46.9		34.7
16	M				53.1	56.5		43.8		49.0		43.3		41.2	
18	F	47.8	43.3		46.9		42.4		45.8		40.4		44.2		55.6
19	F	44.4	44.0		41.2		43.8		46.3		38.4		41.7		44.2
20	F	43.0		39.5		46.3		50.6		41.2		43.3		45.7	
21	F	40.6		54.3		39.2		45.3		50.3		42.5		45.3	
	AVE.	45.8	47.2	45.7	49.3	44.2	45.0	46.8	44.0	51.5	40.7	43.7	44.4	43.2	44.9
GROUP	C														
22	M	49.3	42.0		49.0		47.1		40.0		45.0		45.0		54.9
23	M	51.6		42.0		45.0		30.8		45.6		39.6		41.2	
24	M	50.7		44.2		49.0		52.8		50.0		40.0		42.1	
25	M	51.5	45.5		45.3		58.3		46.7		40.0		42.5		32.0
26	F	47.6		40.0		39.0		45.9		48.6		47.1		41.7	
27	F	48.8	45.5		43.3		43.9		41.0	43.6	40.5		48.4		41.7
28	F	50.4	43.8		42.6		41.2		38.2	51.6	39.5		43.4		47.6
29	F	47.5		40.0		44.7		38.0		52.7		43.5		42.9	
	AVE.	49.7	44.2	41.6	45.1	44.4	47.6	41.9	41.5	48.7	41.2	42.6	44.8	41.9	44.1

TABLE 33 (CONTINUED)

MEAN CELL VOLUME EXPRESSED AS CUBIC MICRONS FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	16	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A																
1	M	39.6		68.9		61.5		51.4		51.3		49.1		44.4		49.0
2	M		39.5		44.2		51.1		41.7		41.7		44.1		44.1	
5	F		42.4		46.8		51.3		51.8		43.7		47.1		49.4	
6	F		40.0		48.0		39.7		45.7		30.9		35.0		43.8	
7	F	42.4		38.5		40.0		50.7		41.0		42.1		48.4		50.0
8	F	40.0		53.8		44.2		52.6		50.5		44.4				49.4
	AVE.	40.7	40.6	53.7	43.0	48.6	47.4	51.6	46.4	47.6	38.8	44.5	42.1	46.4	45.8	49.5
GROUP B																
13	M	43.2		51.4		40.0		52.5		50.5		45.2		42.9		
14	M		43.6		49.5		41.4		40.0		48.0		45.0		48.9	
15	M		36.0		43.8		47.5		43.5		41.5		37.2		44.4	
16	M	38.2		46.7		49.0		54.1		52.9		46.6		49.0		50.0
18	F		39.6		49.5		52.6		38.6		31.6		42.4		43.2	
19	F		35.3		44.1		45.2		47.1		42.1		39.2		41.2	
20	F	42.9		47.1		38.9		50.0		44.6		46.3				50.5
21	F	33.0		48.6		48.0		53.3		50.0		46.5				48.4
	AVE.	39.3	38.6	48.5	46.7	44.0	46.7	52.5	42.3	49.5	40.8	46.2	41.0	46.0	44.4	49.6
GROUP C																
22	M		35.4		45.0		51.1		44.1		40.4		51.5		50.6	
23	M	44.7		53.8		45.5		52.6		51.5		43.6		42.1		
24	M	54.0		35.4		43.0		48.6		51.0		47.2		47.5		50.0
25	M		38.8		44.7		48.4		43.6		41.2		44.0		47.1	
26	F															
27	F		42.2		50.0		50.0		40.3		41.2					
28	F		46.5		46.7		51.6		40.8		42.7		48.3		46.5	
29	F	46.7		32.9		40.0		53.7		42.2		44.4		42.1		50.0
	AVE.	48.5	40.7	40.7	46.6	42.8	50.4	51.6	42.2	48.2	41.4	45.2	47.9	43.9	48.0	50.0

TABLE 34

MEAN CELL HEMOGLOBIN EXPRESSED AS MICROMICROGRAMS/CELL FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP A															
1	M	16.9		15.9		17.3		15.0		13.0		15.7		14.0	
2	M	15.0	12.9		15.6		13.7		14.4		12.7		13.8		14.3
5	F	17.0	15.1		10.0		16.0		15.4		14.9		14.8		15.9
6	F	15.7	12.7		16.2		14.9		14.4		14.4		13.1		14.6
7	F	14.3		16.7		15.0		15.3		15.4		15.4		14.5	
8	F	12.1		14.2		12.5		15.0		9.4		13.6		13.0	
	AVE.	15.2	13.6	17.0	16.9	14.9	14.9	15.1	14.7	12.6	14.0	14.9	13.9	13.8	14.9
GROUP B															
13	M	17.9		12.6		14.9		14.1		16.0		13.0		12.8	
14	M	16.0	17.6		17.9		14.9		13.7		14.7		13.9		14.7
15	M	15.9	16.0		16.9		15.2		13.7		14.7		14.1		13.9
16	M				16.3	15.6		14.3		12.5		14.4		12.8	
18	F	16.3	14.4		16.7		13.6		14.1		14.1		13.3		17.2
19	F	14.8	13.0		14.4	15.6		16.2		13.1		12.9		14.5	
20	F	13.9		13.6		14.4		15.7		12.9		14.2		14.7	
21	F	12.9		16.3		13.7		15.0		13.1		13.5		14.9	
	AVE.	15.4	15.3	14.2	16.4	14.7	14.8	14.8	14.4	13.6	14.2	13.8	13.6	13.7	15.5
GROUP C															
22	M	16.5	13.5		16.2		16.2		13.5		15.4		13.8		17.7
23	M	17.1		14.0		15.0		20.8		14.4		12.4		12.8	
24	M	16.4		15.0		16.2		15.8		14.1		13.1		12.9	
25	M	17.0	13.1		15.1		20.8		16.0		12.6		13.0		10.5
26	F	13.8		13.2		13.6		12.2		8.1		15.6		12.3	
27	F	15.0	13.6		14.4		15.2		14.8	14.5	13.7		15.2		14.8
28	F	16.8	15.6		14.4		14.4		12.9	17.2	13.3		13.4		16.9
29	F	16.9		13.3		14.9		13.0		9.5		14.1		13.5	
	AVE.	16.2	14.0	13.9	15.0	14.9	16.7	15.5	13.0	13.0	13.8	13.8	13.9	12.9	15.0

TABLE 34 (CONTINUED)

MEAN CELL HEMOGLOBIN EXPRESSED AS MICROMICROGRAMS/CELL FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	16	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A																
1	M	14.9		23.3		20.0		16.5		16.9		14.7		13.8		15.0
2	M		13.9		12.9		15.5		12.5		14.1		16.2		14.1	
5	F		14.6		15.4		15.6		14.8		15.1		16.8		15.3	
6	F		13.9		16.0		13.1		17.1		11.8		11.5		13.8	
7	F	14.7		15.4		13.1		16.3		13.9		13.4		15.2		15.3
8	F	16.7		19.2		15.8		16.3		12.4		13.8				15.8
	AVE.	15.4	14.1	19.3	14.8	16.3	14.7	16.4	14.8	14.4	13.7	14.0	14.8	14.5	14.4	15.4
GROUP B																
13	M	16.2		16.0		14.4		16.0		13.6		13.8		13.3		
14	M		15.5		17.3		12.8		11.8		14.8		14.0		15.3	
15	M		12.0		16.9		15.0		14.7		13.9		13.8		13.8	
16	M	15.0		14.9		16.2		17.6		15.2		14.6		15.1		15.2
18	F		13.4		17.0		16.3		10.8		11.5		14.5		13.6	
19	F		11.3		15.2		14.5		16.9		12.6		13.2		13.2	
20	F	13.8		16.2		14.4		15.9		14.4		14.1				16.5
21	F	11.6		16.2		16.0		16.5		15.0		14.1				15.6
	AVE.	14.2	13.1	15.8	16.6	15.3	14.7	16.5	13.6	14.5	13.2	14.2	13.9	14.2	14.0	15.8
GROUP C																
22	M		12.1		17.0		15.5		17.1		14.6		16.8		16.2	
23	M	16.5		19.2		16.6		16.1		11.9		13.6		13.8		15.3
24	M	15.0		12.5		15.6		16.2		12.8		14.5		16.8		15.3
25	M		12.9		14.7		16.5		12.9		13.9		13.5		14.4	
26	F															
27	F	14.5		17.9		15.3		10.3		12.1						
28	F		15.6		16.7		16.1		13.8		15.9		16.1		14.5	
29	F	13.3		16.4		15.6		16.5		13.8		13.6		13.4		15.5
	AVE.	14.9	13.8	16.0	16.6	15.9	15.9	16.3	13.5	12.8	14.2	13.9	15.5	14.6	15.0	15.4

TABLE 35

MEAN CELL CORPUSCULAR HEMOGLOBIN EXPRESSED AS A PERCENTAGE FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP A															
1	M	32.7		34.3		34.4		33.7		25.7		32.1		31.8	
2	M	34.0	32.4		35.7		37.7		34.4		32.2		30.6		30.0
5	F	33.3	32.2		36.2		35.3		33.6		32.6		30.8		37.5
6	F	33.4	22.6		36.4		27.5		38.3		31.0		31.0		31.0
7	F	32.8		32.9		38.6		31.0		36.2		30.8		31.7	
8	F	27.6		33.8		33.3		34.4		20.8		33.3		31.1	
	AVE.	32.3	29.1	33.6	36.1	36.4	32.8	33.0	35.8	27.6	31.9	32.1	30.8	31.5	32.8
GROUP B															
13	M	37.1	31.3	29.2	32.7	33.3	31.0	29.5	32.9	24.4	34.2	28.4	30.9	31.4	30.0
14	M	31.6	31.3	29.2	32.7	33.3	31.0	29.5	32.9	24.4	34.2	28.4	30.9	31.4	30.0
15	M	34.2	35.7		33.4		33.3		32.5		35.7		30.0		40.0
16	M				30.8	33.7		32.6		25.5		33.5		31.0	
18	F	34.1	33.3		35.6		32.1		30.7		35.0		30.0		31.0
19	F	33.4	29.5		35.0		35.7		35.0		34.2		31.0		32.9
20	F	33.0		34.3		31.0		31.1		31.4		32.9		32.1	
21	F	31.2		30.0		35.0		33.1		26.2		31.8		32.9	
	AVE.	33.5	32.5	31.2	33.5	33.3	33.0	31.6	32.8	26.9	34.8	31.9	30.5	31.9	33.5
GROUP C															
22	M	33.4	32.1		33.7		34.4		33.8		34.2		30.1		32.2
23	M	33.2		33.3		35.7		38.3		31.7		31.3		31.0	
24	M	32.3		34.1		33.0		29.9		28.3		32.8		30.6	
25	M	33.0	28.9		33.3		35.7		34.5		31.3		30.6		32.8
26	F	29.1		32.9		35.0		26.7		20.7		33.3		29.6	
27	F	30.9	30.0		33.3		34.7		35.0	33.3	33.8		31.3		35.4
28	F	33.2	35.7		33.8		35.0		33.8	33.3	33.5		30.9		35.5
29	F	35.6		33.3		33.3		34.2		17.9		32.5		31.4	
	AVE.	32.6	31.7	33.4	33.5	34.3	35.0	32.3	34.3	27.5	33.2	32.6	30.9	30.7	34.0

TABLE 35 (CONTINUED)

MEAN CELL CORPUSCULAR HEMOGLOBIN EXPRESSED AS A PERCENTAGE FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	16	17	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP	A															
1	M	37.5		33.8		32.6		32.1		32.9		31.0		31.0		30.8
2	M		35.3		37.7		30.2		30.0		33.8		36.7		32.0	
5	F		34.5		33.0		30.5		28.6		34.7		35.8		31.0	
6	F		34.7		33.0		33.0		37.5		38.2		32.9		31.4	
7	F	34.7		40.0		32.7		32.2		33.8		31.2		31.3		30.6
8	F	41.7		35.7		35.7		31.0		24.5		31.0				32.1
	AVE.	38.0	34.8	35.6	34.6	33.6	31.2	31.8	32.0	30.4	35.6	31.1	33.9	31.2	31.5	31.2
GROUP	B															
13	M	37.5		31.1		36.1		30.5		27.0		30.2		31.1		
14	M		35.4		35.0		30.8		29.4		30.8		31.1		31.3	
15	M		33.3		38.6		31.6		33.8		33.3		37.1		31.0	
16	M	30.6		32.0		33.0		32.6		28.7		31.0		30.8		30.5
18	F		33.6		34.4		31.0		27.9		36.7		34.2		31.4	
19	F		31.9		34.4		32.1		36.0		30.0		33.8		32.0	
20	F	32.1		34.4		37.1		31.9		32.2		30.1				32.6
21	F	35.1		33.3		33.2		31.0		30.0		30.1				32.1
	AVE.	33.8	33.6	33.0	35.6	34.9	31.4	31.5	31.8	29.5	32.7	30.1	34.1	31.0	31.4	31.7
GROUP	C															
22	M		34.3		37.8		30.2		38.1		36.5		32.7		32.0	
23	M	36.8		35.7		36.7		30.5		23.0		31.0		32.5		
24	M	44.1		35.4		36.3		33.3		27.0		30.1		35.4		31.3
25	M		33.3		32.9		34.0		29.5		33.8		30.7		30.5	
26	F															
27	F		34.3		35.7		30.6		25.7		30.0					
28	F		33.6		35.7		31.3		33.8		37.1		33.3		31.3	
29	F	28.6		50.0		38.9		30.7		32.6		30.0		31.9		31.0
	AVE.	36.5	33.9	40.4	35.5	37.3	31.5	31.5	31.9	27.5	34.4	30.0	32.2	33.3	31.3	31.2

TABLE 36

TOTAL SERUM PROTEIN CONCENTRATION EXPRESSED AS gms/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17
GROUP A																	
1	M	6.3		5.8		6.0		6.8		6.0		6.4		6.0		6.0	
2	M	6.8	6.6		6.6		6.4		7.0		8.0		7.2		6.4		6.5
5	F	7.1	7.0		7.0		6.2		6.8		7.2		7.6		5.0		7.2
6	F	9.4	7.2		7.4		8.8		9.0		9.0		8.6		8.6		9.0
7	F	7.8		7.4		8.0		7.6		7.0		6.8		8.0		7.8	
8	F	7.2		7.5		7.2		7.4		7.2		7.0		6.0		7.6	
	AVE.	7.4	6.9	6.9	7.0	7.1	7.1	7.3	7.6	6.7	8.1	6.7	7.8	6.7	6.7	7.1	7.6
GROUP B																	
13	M	6.5		7.2		7.2		7.6		6.2		6.2		6.5		6.5	
14	M	7.1	7.6		8.6		7.2		7.2		7.2		7.4		7.2		7.8
15	M	7.2	6.2		7.0		6.8		6.6		7.2		6.6		7.4		7.0
16	M				7.2	6.4		6.6		6.2		6.2	6.0	6.0		7.4	
18	F	6.6	6.8		6.0		6.2		6.6		6.2		6.2		7.0		6.2
19	F	5.9	6.4		6.0		6.0		7.0		6.8		6.0		6.4		6.0
20	F	7.5		6.0		7.5		6.5		6.6		6.4		7.6		7.4	
21	F	7.1		7.0		6.5		5.0		6.4		6.8		7.4		8.4	
	AVE.	6.8	6.8	6.7	7.0	6.9	6.6	6.4	6.9	6.4	6.9	6.4	6.6	6.9	7.0	7.4	6.8
GROUP C																	
22	M	6.7	6.8		8.0		5.2		7.0		8.0		7.0		7.0		5.8
23	M	8.2		8.0		8.4		7.4		6.2		6.8		8.0		7.8	
24	M	7.3		6.5		7.8		8.0		7.4		6.4		6.4		7.2	
25	M	7.8	7.4		6.8		7.0		7.0		6.6		6.2		6.3		6.4
26	F	7.2		7.4		4.4		6.5		6.2		7.8		6.4			
27	F	8.0	7.0		7.0		7.0		6.8	7.0	7.0		5.8		6.8		7.0
28	F	7.2	7.0		7.4		7.8		7.0	6.2	7.2		7.2		7.0		7.5
29	F	7.8		8.4		8.2		7.8		6.5		8.0		9.0		8.4	
	AVE.	7.5	7.1	7.6	7.3	7.2	6.8	7.4	7.0	6.6	7.2	7.3	6.6	7.5	6.8	7.8	6.7

TABLE 36 (CONTINUED)

TOTAL SERUM PROTEIN CONCENTRATION EXPRESSED AS gms/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A														
1	M	6.2		6.0		5.5		6.4		6.2		6.8		6.2
2	M		6.4		6.6		6.5		7.0		7.6		7.2	
5	F		7.2		7.0		7.8		8.0		7.2		7.0	
6	F		8.0		8.0		9.8		9.0		9.5		8.0	
7	F	7.0		7.6		8.0		7.0		6.0		7.5		7.5
8	F	7.5		8.0		8.0		7.0		7.0				8.0
	AVE.	6.9	7.2	7.2	7.2	7.2	8.0	6.8	8.0	6.4	8.1	7.2	7.4	7.2
GROUP B														
13	M	7.2		7.2		6.4		7.0		6.6		7.0		
14	M		8.0		7.4		7.2		7.8		7.2		7.2	
15	M		7.0		6.4		6.8		7.8		7.5		6.5	
16	M	7.0		7.0		6.6		7.4		7.5		6.5		7.0
18	F		7.6		6.6		6.5		6.5		6.6		6.2	
19	F		6.2		6.8		7.8		6.5		8.0		6.5	
20	F	8.0		7.0		6.4		7.5		6.8				7.0
21	F	7.0		6.5		6.2		7.2		7.2				7.0
	AVE.	7.3	7.2	6.9	6.8	6.4	7.1	7.3	7.2	7.0	7.3	6.8	6.6	7.0
GROUP C														
22	M		8.2		8.0		7.[illegible]		7.2		8.0		6.2	
23	M	7.0		7.2		9.0		7.8		8.0		9.0		
24	M	6.5		7.2		6.6		7.0		6.8		7.0		7.0
25	M		7.0		6.6		6.5		6.5		7.2		7.6	
26	F		7.0		6.6									
27	F		7.0		6.6		8.0		5.5					
28	F		7.0		6.8		7.5		7.0		7.8		7.0	
29	F	8.2		8.5		9.0		8.4		7.6		8.2		8.0
	AVE.	7.2	7.3	7.6	6.8	8.2	7.3	7.7	6.6	7.5	7.7	8.1	6.9	7.5

TABLE 37

SERUM ALBUMIN CONCENTRATION EXPRESSED AS gm/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 0	1	2	3	4	5	6	7	8	20
GROUP A											
1	M	3.1		2.8		2.5			3.4		
2	M						3.2		3.6		
5	F	3.5			2.5		3.0				
6	F	3.0					3.8		4.2		
7	F			3.5		3.6				3.6	2.7
8	F			1.4		3.1				3.8	2.2
	AVE.	3.2		2.6	2.5	3.1	3.3		3.7	3.7	2.5
GROUP B											
13	M	3.1		2.8		3.5			3.6		2.8
14	M	3.5			4.1		3.4		3.8		
15	M				4.0		3.4		3.6		
16	M				3.7	3.3			3.8		3.8
18	F				3.0		3.0		3.4		
19	F				3.0		3.0		3.8		
20	F			2.8						3.2	2.8
21	F			2.8						3.2	1.8
	AVE.	3.0		2.8	3.6	3.4	3.2		3.7	3.2	2.8
GROUP C											
22	M	3.5			4.2		2.6		3.6		
23	M					2.2				3.0	2.5
24	M			3.5		4.0				3.4	4.5
25	M				3.2		3.4		3.6		
26	F			3.1		3.3				3.0	
27	F				3.5		3.5		3.4	3.2	
28	F	3.6			2.5		3.8		3.8	2.8	
29	F	3.0		2.5		3.5				3.0	2.8
	AVE.	3.4		3.0	3.4	3.3	3.3		3.6	3.1	3.3

TABLE 37 (CONTINUED)

SERUM ALBUMIN CONCENTRATION EXPRESSED AS gm/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	21	22	23	24	25	27	30	31	35	38
GROUP A											
1	M		2.6		3.0		3.4				3.4
2	M	3.1		2.5		2.6				3.8	
5	F	2.5		3.3		3.1		3.2		4.3	
6	F	3.0		3.3		2.1		3.5		4.0	
7	F		4.0		2.5		3.5				3.8
8	F		4.2		2.6		3.8				4.0
	AVE.	2.9	3.6	3.0	2.7	2.6	3.6	3.4		4.0	3.7
GROUP B											
13	M		3.0		3.4		3.6				
14	M	3.6		3.5		4.0		3.5		3.8	
15	M	3.1		3.1		2.6	3.6				
16	M		3.2		3.5			3.2		3.3	
18	F	2.5		2.5		2.5		3.2		3.3	
19	F	2.5				3.4		4.2		3.7	
20	F		3.0		3.1		3.4				3.6
21	F		3.0		2.8		3.4				3.6
	AVE.	2.9	3.1	3.0	3.2	3.1	3.5	3.5		3.5	3.6
GROUP C											
22	M	2.5		3.3		3.6		4.4		3.3	
23	M		4.4		2.8		3.6				
24	M		3.2		3.3		3.5				3.8
25	M	2.3				3.4		3.4		3.8	
26	F										
27	F	3.3		3.0		2.8		3.6		3.8	
28	F	3.2		3.2		3.6					
29	F		4.2		2.2		3.6				3.8
	AVE.	2.8	3.9	3.2	2.8	3.4	3.6	3.8		3.6	3.8

TABLE 38

SERUM GLOBULIN CONCENTRATION EXPRESSED AS gm/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	20
GROUP A											
1	M	3.2		3.0		3.5				2.6	
2	M						3.2		3.4		
5	F	4.5			4.5		3.2		3.2		
6	F	6.8					5.0		4.8		
7	F			3.9		4.4				3.4	4.9
8	F			6.1		4.1				3.4	5.8
	AVE.	4.8		3.5	4.5	4.0	3.8		3.8	3.1	5.4
GROUP B											
13	M	3.0		4.4		3.7				2.6	4.4
14	M	3.8			4.5		3.8		3.1		
15	M				3.0		3.4		3.0		
16	M				3.5	3.1				2.4	4.2
18	F				3.0		3.2		3.2		
19	F				3.0		3.0		3.2		
20	F			3.2						3.4	4.2
21	F			4.2						3.2	4.7
	AVE.	3.4		3.9	3.4	3.4	3.4		3.1	2.9	4.4
GROUP C											
22	M	3.1			3.8		2.6		3.4		
23	M			5.8						3.2	4.7
24	M			3.0		3.8				3.0	2.7
25	M				2.6		3.6		3.4		
26	F			4.3		1.1				3.3	
27	F				3.5		3.5		3.4	3.8	
28	F	3.5			4.9		4.0		3.2	3.4	
29	F	5.0		5.9		4.7				3.5	5.7
	AVE.	3.9		4.8	3.7	3.2	3.4		3.4	3.4	4.4

TABLE 38 (CONTINUED)

SERUM GLOBULIN CONCENTRATION EXPRESSED AS gm/100 cc FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	21	22	23	24	25	27	30	31	35	38
GROUP A											
1	M		2.9		3.4		2.8				2.8
2	M	3.5		4.0		4.4				3.4	
5	F	4.5		4.5		4.9		3.2		4.3	
6	F	5.0		6.5		6.9		6.0		4.0	
7	F		4.0		4.5		2.5				3.7
8	F		3.8		4.4		3.2				4.0
	AVE.	4.3	3.9	5.0	4.1	5.4	2.8	4.6		3.9	3.5
GROUP B											
13	M		3.4		3.6		3.4				
14	M	3.8		3.7		3.8					
15	M	3.3		3.7		5.2					
16	M		3.4		3.9		3.4				
18	F	4.1		4.0		4.0					
19	F	3.3				3.1					
20	F		3.4		4.4		3.4				
21	F		3.2		4.4		3.8				
	AVE.	3.6	3.3	3.8	4.4	4.0	3.5	3.8		3.1	3.4
GROUP C											
22	M	5.5		3.7		3.6					
23	M		4.6		5.0		4.4				
24	M		3.4		3.7		3.6				
25	M	3.3				3.1					
26	F										
27	F	3.3		5.0		2.7					
28	F	3.6		4.3		3.4					
29	F		4.8		6.2		4.0				
	AVE.	3.7	4.3	4.3	5.0	3.2	4.0	3.9		3.4	3.7

TABLE 39

SERUM INORGANIC PHOSPHATE LEVELS EXPRESSED AS mg/100 ml FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17
GROUP	A																
1	M	4.1		4.5		5.8		5.4		5.0		5.0		4.3		4.1	
2	M	5.0	4.0		4.5		4.9		5.4		3.4		3.8		4.5		4.5
5	F	4.0	4.1		4.1		4.2		4.5		4.5		4.0				4.1
6	F	5.1	4.0				4.3		4.3		4.6		3.6		4.5		4.3
7	F	5.2		4.1		4.4		4.3		5.0		4.5		4.9		5.0	
8	F	3.7		3.6		4.2		4.0		4.3		3.4		4.3		3.1	
	AVE.	4.5	4.0	4.1	4.3	4.8	4.5	4.6	4.7	4.8	4.2	4.3	3.8	4.5	4.5	4.1	4.3
GROUP	B																
13	M	4.2		4.3		5.8		5.8		5.8		4.7		4.9		5.1	
14	M	4.5	4.8		5.4		5.8		5.2		5.5		5.2		5.0		4.3
15	M	5.5	4.3		4.7		5.4		5.6		5.2		4.7		5.4		4.9
16	M				4.1	5.1		5.8		5.0		5.0		5.0		5.4	
18	F	5.0	3.6		4.4		5.2		5.0		4.5		4.0		4.5		4.3
19	F	4.7	4.5		4.0		4.9		5.4		4.0		4.5		3.6		4.5
20	F	4.6		4.0		5.4		4.5		5.2		4.3		4.8		4.1	
21	F	4.2		3.6						4.7		4.7		5.4		4.0	
	AVE.	4.7	4.3	4.0	4.5	5.4	5.3	5.4	5.3	5.2	4.8	4.7	4.6	5.0	4.6	4.6	4.5
GROUP	C																
22	M	4.2	4.7		4.3		5.6		4.5		5.5		5.0		5.4		-
23	M	4.8		4.1		4.4		4.3		4.3		4.3		4.3		4.9	
24	M	4.8		3.6		4.9		6.1		4.2		4.1		3.6		4.0	
25	M	5.5	5.0		5.0		5.6		5.6		4.5		4.7		4.7		4.5
26	F	4.4		5.0		4.4		4.3		4.9		4.3		4.1			
27	F	5.2	4.8		4.7		5.0		5.7	5.4	5.1		5.0		4.1		4.7
28	F	3.7	4.0		4.0		4.9		4.5	5.8	5.7		4.0		4.3		4.1
29	F	4.1		3.4		4.4		4.3		5.2		4.0		4.9		4.3	
	AVE.	4.6	4.6	4.0	4.5	4.5	5.3	4.8	5.1	5.0	5.2	4.2	4.7	4.2	4.6	4.4	4.4

TABLE 39 (CONTINUED)

SERUM INORGANIC PHOSPHATE LEVELS EXPRESSED AS mg/100 ml FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A														
1	M	4.5		4.2		4.5		3.8		4.0				4.2
2	M		3.8		4.7		4.0		3.6		3.6		4.0	
5	F		4.5		5.0		4.1		3.8		3.5		3.6	
6	F		4.0		3.6		4.1		3.8		4.5		4.0	
7	F	4.0		3.6		4.0		4.0		3.6				3.4
8	F	3.2		4.3		4.3		3.8		3.4				5.4
	AVE.	3.9	4.1	4.0	4.4	4.3	4.1	3.9	3.7	3.5	3.9		3.9	4.3
GROUP B														
13	M	4.1		4.1		4.4		4.7						
14	M		4.9		4.9		5.0		4.0		3.8		4.0	
15	M		3.4		4.3		4.9		4.0		4.3		4.0	
16	M	4.5		4.3		4.6		3.8		4.0				5.1
18	F		4.3		4.7		4.5		4.0		3.5		3.6	
19	F		3.8		4.0		4.3		3.3		3.5		3.6	
20	F	4.3		4.0		4.4		4.7		4.0				4.4
21	F			4.5		4.6		4.7		4.0				5.1
	AVE.	4.3	4.1	4.2	4.5	4.5	4.7	4.5	3.8	4.1	3.8		3.8	4.8
GROUP C														
22	M		5.4		4.7		4.9		3.8		4.1		4.0	
23	M	4.1		4.0		4.7		4.9		3.6				
24	M	4.3		4.2		4.6		4.3		3.6	4.1			3.6
25	M		4.3		4.3		4.9		3.3		3.5		3.6	
26	F													
27	F		4.3		4.9		4.1		3.6					
28	F		4.5		4.7		4.3		3.4		3.6		3.6	
29	F	4.3		4.2		4.7		4.0		4.1				3.6
	AVE.	4.2	4.6	4.1	4.7	4.7	4.6	4.4	3.5	3.8	3.8		3.7	3.6

TABLE 40

SERUM INORGANIC CALCIUM EXPRESSED AS mEq/1 FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17
GROUP	A																
1	M	4.8		3.8		2.9		3.9		4.7		4.3		4.6		4.5	
2	M	4.6	4.5		3.8		3.9		4.6		4.6		4.5		3.7		4.1
5	F	4.4			4.0		4.1		4.8		4.4		4.4				4.4
6	F	4.6	4.2		4.0		4.0		4.6		3.6		4.4		3.8		4.2
7	F	4.7		3.8		4.3		2.4		4.4		4.0		4.6		4.6	
8	F	4.5				4.4		3.9		4.4		3.8		4.4		4.1	
	AVE.	4.6	4.4	3.8	3.9	3.5	4.0	3.4	4.7	4.5	4.2	4.0	4.4	4.5	3.8	4.4	4.2
GROUP	B																
13	M	4.0		3.9		4.7		4.0		4.3		4.0		4.5		4.2	
14	M	4.6	4.7		4.5		4.3		4.8		4.0		4.2		4.0		4.5
15	M	4.7	4.1		4.6		4.5		4.8		4.2		4.6		4.6		4.1
16	M					4.0		4.1		4.3		4.2		4.6		4.1	
18	F	4.7	4.4		4.3		4.2		4.7		4.6		4.4		3.8		4.3
19	F	4.6	4.2		3.9		4.1		4.6		4.2		4.5		4.1		4.1
20	F	4.2		3.5		3.4		2.9		4.4		3.9		4.4		4.1	
21	F			3.6						4.1		3.9		4.5		4.2	
	AVE.	4.5	4.4	3.7	4.3	4.0	4.3	3.7	4.7	4.3	4.3	4.0	4.4	4.5	4.1	4.2	4.3
GROUP	C																
22	M	4.7	4.5		4.3		4.2		4.6		4.2		4.4		3.8		
23	M	4.7		3.9		4.4		3.8		4.3		4.1		4.1		4.1	
24	M	4.6		3.9		3.8		4.5		4.8		4.0		4.2		4.2	
25	M	4.7	4.6		4.3		4.3		4.6				4.4		4.0		4.1
26	F	3.8				2.7		3.8		4.2		4.0		4.2			
27	F	4.7	4.5		4.0		4.3		4.5	4.2	4.1		4.4		4.2		4.4
28	F	4.6	4.5		4.4		4.5		4.5	4.1	4.1		4.0		4.1		4.1
29	F	4.8		3.7		4.9		3.9		4.6		4.1		4.0		4.4	
	AVE.	4.6	4.5	3.8	4.3	4.0	4.3	4.0	4.6	4.4	4.1	4.1	4.3	4.1	4.0	4.2	4.2

TABLE 40 (CONTINUED)

SERUM INORGANIC CALCIUM EXPRESSED AS mEq/1 FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	18	19	20	21	22	23	24	25	27	30	31	35	38
GROUP A														
1	M			3.5				4.5		4.7				4.5
2	M		4.0		4.3		4.0		4.2		4.5		4.7	
5	F		3.9		3.8		4.3		4.8		4.8		4.6	
6	F		-		4.4		4.0				4.8		4.5	
7	F	4.2		3.7		4.3		4.5		4.7				4.0
8	F			4.2		4.0		4.3		4.4				4.7
	AVE.	4.2	3.0	3.8	4.2	4.2	4.1	4.4	4.5	4.6	4.7		4.6	4.4
GROUP B														
13	M			4.0		3.7		4.6		4.8				
14	M		3.5		4.4		4.5		4.5		4.5		4.8	
15	M		3.8		4.3		4.1		4.2		4.6		4.6	
16	M	4.4		4.2		3.8		4.4		4.7				4.5
18	F		3.8		4.2		4.4		4.4		4.6		4.4	
19	F		4.2		4.0				4.5		4.6		4.5	
20	F	4.2		4.0		3.9		4.8		4.6				4.1
21	F			4.3				4.4		4.6				3.7
	AVE.	4.3	3.7	4.1	4.2	3.8	4.3	4.6	4.4	4.7	4.6		4.8	4.1
GROUP C														
22	M		3.9		3.8		4.8		4.7		4.5		4.5	
23	M			3.8		3.7		4.6		4.4				
24	M	4.4		4.0		3.7		4.4		4.4	4.7			4.5
25	M		4.0		3.6				4.3		4.6		4.4	
26	F													
27	F		3.9		4.1		4.4		4.3					
28	F		3.7		4.2		4.7		4.4		4.6		4.5	
29	F	4.0		4.0		3.8		4.4		4.7				4.5
	AVE.	4.2	3.9	3.9	3.9	3.7	4.6	4.5	4.4	4.5	4.6		4.5	4.5

TABLE 41

SERUM CHLORIDE LEVELS EXPRESSED AS mEq/1 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 0	1	2	3	4	5	6	7	8
GROUP A										
1	M	116.1		122.6		117.0		117.9		119.8
2	M	116.6	128.5		120.7		117.2		113.6	
5	F	117.4			118.3		121.5		114.8	
6	F	123.9	120.5				114.0		115.2	
7	F	115.0		120.4		117.0		118.9		124.2
8	F	127.9				114.9		121.1		114.3
	AVE.	119.5	124.5	121.5	119.5	116.3	117.6	119.4	114.5	119.4
GROUP B										
13	M	118.1		113.9		113.8		111.6		121.3
14	M	116.0	115.9		115.0				114.5	
15	M	117.2	112.0		110.3		117.2		114.4	
16	M					121.3		113.7		116.5
18	F	118.6	129.5		125.8		112.9		116.2	
19	F	120.0	122.7		122.6		119.4		114.5	
20	F	126.0		115.1		113.8		116.8		118.6
21	F			119.4						118.6
	AVE.	119.3	120.0	116.1	118.4	116.3	116.5	114.0	114.9	118.8
GROUP C										
22	M	119.8	125.0		114.0		117.2		113.8	
23	M	117.5		118.3		117.0		113.7		121.9
24	M	117.7		118.3				110.5		109.9
25	M	114.2	114.8		115.0		120.4		114.2	
26	F	126.7				124.5		116.8		116.5
27	F	124.4	128.6		118.3		118.3		112.8	120.9
28	F	115.0	121.6		116.4	112.8	118.3		112.8	116.5
29	F	119.0		121.6		112.8		116.8		115.5
	AVE.	119.3	122.5	119.4	115.9	118.1	118.6	114.5	113.4	116.9

TABLE 41 (CONTINUED)

SERUM CHLORIDE LEVELS EXPRESSED AS mEq/1 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	9	10	11	12	13	16	17	18	19	20
GROUP A											
1	M		120.8		119.4		118.4				113.1
2	M	122.8		118.8		117.0		117.9		116.8	
5	F	127.1		119.8				120.0			
6	F	117.4		118.6		119.2		116.6			
7	F		120.8		120.5		115.3		117.5		115.5
8	F		125.0		118.5		115.3				118.2
	AVE.	122.4	122.2	119.1	119.5	118.1	116.3	116.5	117.5	116.8	115.6
GROUP B											
13	M		120.8		118.4		117.3				117.4
14	M	119.6		118.8		117.0		117.9		113.8	
15	M	123.9		116.6		119.4		116.8		116.2	
16	M		118.8		117.8		114.3		118.4		120.0
18	F	126.1		118.0		116.0		116.8		114.9	
19	F	127.2		116.8		120.2		118.9			
20	F		120.8		121.0		116.3		119.0		117.4
21	F		116.7		119.2		114.3				119.5
	AVE.	124.3	119.3	117.6	119.1	118.2	115.6	117.6	118.7	114.9	118.6
GROUP C											
22	M	115.2		117.5		117.0				112.8	
23	M		116.7		119.5		111.2				116.0
24	M		116.7		116.8		116.3		118.0		117.4
25	M	121.7		119.5		118.2		118.4		111.1	
26	F		125.0		116.2						
27	F	120.9		117.5		122.5		118.9		117.2	
28	F	122.8		122.5		117.0	118.4		117.8		117.4
29	F		118.8		120.0			115.8		116.2	
	AVE.	120.2	119.3	119.3	118.2	118.7	115.3	117.9	117.9	114.3	116.9

TABLE 41 (CONTINUED)

SERUM CHLORIDE LEVELS EXPRESSED AS mEq/1 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	21	22	23	24	25	27	30	31	35	38
GROUP A											
1	M				116.7		117.1				116.0
2	M	118.6		117.0		120.8		114.4		117.5	
5	F	118.4		107.4		122.9		114.4		117.2	
6	F	116.0		110.0				112.4		117.0	
7	F		116.0		118.0		120.0				114.0
8	F		112.8		123.4		118.1				120.0
	AVE.	117.7	114.4	111.5	119.4	121.9	119.1	113.7		117.2	116.7
GROUP B											
13	M		118.2		121.3		118.5				
14	M	115.8		112.0		119.8		116.6		117.5	
15	M	117.8		111.0		116.7		112.4		117.2	
16	M		119.0		119.1		120.0				
18	F	119.0		112.0		118.8		112.4		116.5	
19	F	116.4				119.8		114.4		116.4	
20	F		118.2		122.9		118.5				114.0
21	F				119.1		117.0				110.0
	AVE.	117.3	118.5	111.7	120.6	118.8	118.3	114.0		116.9	112.0
GROUP C											
22	M	119.2		112.0		119.8		114.4		116.5	
23	M		114.4		119.1		116.0				
24	M		118.2		125.5		120.0				111.0
25	M	117.0				120.2		116.6		116.5	
26	F										
27	F	117.5		114.0		121.5					
28	F		114.4		120.8		118.0	116.6		117.5	
29	F	117.2		117.0		120.2					114.0
	AVE.	117.7	115.7	114.3	121.8	120.4	118.0	115.9		116.8	112.5

TABLE 42

TOTAL LEUCOCYTE COUNT EXPRESSED AS NUMBER OF LEUCOCYTES/mm^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 0	1	2	3	4	5	6	7	8	9
GROUP A											
1	M	14.1		15.6		12.1		10.4		11.8	
2	M	16.0	11.1		10.1		9.8		12.6		15.4
5	F	11.8	15.6		14.6		10.0		11.2		9.4
6	F	14.7	20.1		14.2		10.1		10.4		13.3
7	F	14.9		16.8		9.9		9.2		11.4	
8	F	7.4		12.6		12.1		10.5		8.6	
	AVE.	13.3	15.6	15.0	13.0	11.4	10.0	10.0	11.4	10.6	12.7
GROUP B											
13	M	15.1		19.7		13.3		21.1		10.1	
14	M	16.3	9.1		9.0		13.1		12.3		11.1
15	M	16.9	8.7		15.8		11.8		15.8		9.0
16	M				17.1	18.8		16.7		12.8	
18	F	14.5	14.6		15.4		11.8		15.2		10.2
19	F	13.7	13.4		9.2		8.0		10.4		10.6
20	F	11.5		12.1		14.6		11.8		16.1	
21	F	23.3		14.2		18.8		22.5		17.7	
	AVE.	15.9	11.5	15.3	13.3	16.4	11.2	18.0	13.4	14.2	10.2
GROUP C											
22	M	13.4	16.7		16.4		11.4		11.8		16.1
23	M	14.5		15.5		8.8		14.4		9.8	
24	M	18.7		9.8		16.4		18.8		12.8	
25	M	16.7	20.1		11.2		12.4		13.8		11.8
26	F	25.6		18.8		9.2		16.0		19.8	
27	F	11.2	13.6		15.5		20.6		10.6	16.0	9.1
28	F	10.8	13.7		11.4		17.7		18.1	14.4	9.2
29	F	16.3		14.7		16.2		10.1		8.0	
	AVE.	15.8	16.0	14.7	13.6	12.7	15.5	14.8	13.6	13.5	11.6

TABLE 42 (CONTINUED)

TOTAL LEUCOCYTE COUNT EXPRESSED AS NUMBER OF LEUCOCYTES/mm^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 10	11	12	13	16	17	18	19	20	21
GROUP A											
1	M	15.5		13.3		15.2		25.0		12.0	
2	M		14.8		16.2		13.6		20.0		12.5
5	F		17.2		9.7		10.0		8.8		12.2
6	F		15.5		9.1		14.4		13.0		15.8
7	F	9.8		9.9		8.8		11.1		8.9	
8	F	8.5		12.6		8.9		9.8		14.2	
	AVE.	11.3	15.8	11.9	11.7	11.0	12.7	10.5	13.9	11.7	13.5
GROUP B											
13	M	14.8		15.6	16.6	12.6	12.4	10.0	11.2	18.4	14.6
14	M		10.1		16.6		12.4		11.2		14.6
15	M		12.2		12.4		15.8		14.2		15.1
16	M	14.2		11.5		11.6		17.7		15.5	
18	F		10.6		12.2		17.6		14.2		15.5
19	F		15.9		13.1		14.4		12.0		21.2
20	F	11.2		10.4		13.8		13.3		12.4	
21	F	12.8		16.2		26.5		42.0		19.2	
	AVE.	13.3	12.4	13.4	13.6	16.1	15.1	23.0	12.9	16.4	16.6
GROUP C											
22	M		16.6		15.4		17.1		18.2		16.0
23	M	8.8		12.1		13.5		9.5		8.9	
24	M	14.1		11.1		11.2		15.8		16.1	
25	M		16.3		14.0		11.2		14.0		16.2
26	F	16.2		17.5							
27	F		13.4		9.8		13.0		6.0		15.7
28	F		18.5		14.8		11.8		11.0		15.5
29	F	13.2		18.1		18.8		19.9		18.1	
	AVE.	13.1	16.2	14.8	13.5	14.5	13.3	15.1	12.3	14.4	15.9

TABLE 42 (CONTINUED)

TOTAL LEUCOCYTE COUNT EXPRESSED AS NUMBER OF LEUCOCYTES/mm^3 FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 22	23	24	25	27	30	31	35	38
GROUP A										
1	M	10.8		15.8		11.7		12.8		12.2
2	M		10.1		14.4		15.0		10.2	
5	F		9.8		14.6		10.1		11.6	
6	F		14.2		20.1		18.8		17.8	
7	F	12.2		10.6		12.4		13.7		12.8
8	F	11.5		11.4		11.4				11.1
	AVE.	11.5	11.4	12.6	16.4	11.8	14.6	13.3	13.2	12.0
GROUP B										
13	M	14.3	14.8	12.1	12.0	12.4		14.4		
14	M		14.8		12.0		14.5		14.1	
15	M		13.8		12.2		12.8		13.4	
16	M	14.7		15.5		11.6		14.1		12.4
18	F		13.8		18.8		11.1		15.5	
19	F		9.2		14.1		9.2		9.8	
20	F	14.5		13.2		12.8				13.3
21	F	13.8		18.2		13.2				12.4
	AVE.	14.3	12.9	14.8	14.3	12.5	11.9	14.2	13.2	12.7
GROUP C										
22	M		16.2		18.9		14.1		10.3	
23	M	15.5		11.0		11.9		12.8		
24	M	10.2		9.5		13.1		11.4		11.6
25	M		10.4		15.4		14.0		8.9	
26	F									
27	F		9.4		9.8					
28	F		12.4		9.8		11.1		12.6	
29	F	15.8		13.0		12.5		17.5		15.5
	AVE.	15.8	12.1	11.2	13.5	12.5	13.1	13.9	10.6	13.6

TABLE 43

BLOOD UREA NITROGEN VALUES EXPRESSED AS mg/100 ml FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	22		20		30		30		25		25		25		20		15	
2	M	25	30		30		20		30		10		25		25		20		30
5	F	24	25		25		15		25		15		25		10		35		25
6	F	30	30		10		25		30		10		25		25		30		-
7	F	25		20		25		20		25		10		25		25		20	
8	F	30		20		25		35		25		-		25		25		30	
	AVE.	26	28	20	22	27	20	28	28	25	12	18	25	25	20	23	28	22	28
GROUP B																			
13	M	26		20		30		20		15		15		20		20		25	
14	M	26	30		15		20		30		15		25		10		20		25
15	M	30	15		30		30		20		10		25		30		25		15
16	M				15	30		20		30		15		25		25		20	
18	F	25	25		20		30		25		20		20		30		25		30
19	F	25	25		30		35		20		25		20		25		20		-
20	F	25		25		25		25		30		-		30		20		20	
21	F	25		20		20		10		20		-		25		20		-	
	AVE.	26	24	22	22	26	29	21	24	24	18	15	23	25	24	21	23	22	23
GROUP C																			
22	M	23	30		10		25		30		10		15		10		-		25
23	M	30		35		35		30		30		15		30		35		30	
24	M	25		25		30		30		30		15		20		25		25	
25	M	30	30		30		30		30		25		30		25		25		25
26	F	20		25		20		25		25		-		20					
27	F	25	10		10		15		15	15	-		10		20		15		25
28	F	24	30		25		20		20	20	10		15		15		25		20
29	F	25		25		30		25		25				30		20		30	
	AVE.	25	25	28	19	29	23	28	24	24	15	15	18	25	18	27	22	28	32

TABLE 43 (CONTINUED)

BLOOD UREA NITROGEN VALUES EXPRESSED AS mg/100 ml FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

		WEEKS POST-INFECTION										
CAT #	SEX	20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	15		20		15		20				25
2	M		20		20		15				20	
5	F		25		25		20		30		25	
6	F		25		25		20		25		20	
7	F	25		25		15		15				20
8	F	30		20		15		20				30
	AVE.	23	23	22	23	15	18	18	28	-	22	25
GROUP B												
13	M	25		25		20		25				
14	M		25		20		25		25		25	
15	M		15		25		20		20		20	
16	M	25		25		25		25				30
18	F		25		20		15		25		25	
19	F		20		10		25		20		20	
20	F	30		30		20		20				20
21	F	20		20		15		20				25
	AVE.	25	21	25	19	20	21	23	23	-	23	25
GROUP C												
22	M		10		20		20		25		20	
23	M	45		30		30		25				30
24	M	30		30		15		25		20		
25	M		25		10		30		25		20	
26	F											
27	F		20		20		20					
28	F		20		15		20		20		20	
29	F	20		35		20		25				30
	AVE.	32	19	32	22	22	23	25	23	20	20	30

TABLE 44

SERUM GLUCOSE VALUES EXPRESSED AS mg/100 ml FOR
EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

WEEKS POST-INFECTION

CAT #	SEX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	16	17	18	19
GROUP A																			
1	M	96				65		65		65		90		90		65		45	
2	M	105	90				90		90		65		80		90		110		110
5	F	79	90		90		65		65		65		65		65		80		90
6	F	73	90		110		90		90		90		65		90		90		90
7	F	73				45		45		65		80		80		90		90	
8	F	75				65		65		45		90		90		90		65	
	AVE.	84	90	-	100	58	82	58	82	58	73	87	70	87	82	82	93	67	97
GROUP B																			
13	M	85				90		65		65		80		80		90		30	
14	M	90	65		90		65		65		90		80		65		90		65
15	M	70	65		90		90		90		65		90		65		80		65
16	M				90	90		65		45		90		90		110		65	
18	F	82	90		65		65		90		80		80		80		90		90
19	F	73	65		90		90		90		90		65		65		110		65
20	F	65				45		45		65		80		65		65		110	
21	F	68				65		65		45		90		90		65		65	
	AVE.	76	71		85	73	78	60	84	55	81	85	79	81	69	83	93	80	71
GROUP C																			
22	M	95	65		65		65		65		80		110		65		90		90
23	M	110				65		90		45		90		65		110		65	
24	M	80				65		65		45		90		80		110		120	
25	M	90	65		90		65		90		90		90		90		65		65
26	F	80				65		65		65		90		65					
27	F	90	90		65		90		90	45	90		65		90		110		90
28	F	87	90		90		90		90	45	90		90		80		90		65
29	F	90				90		65		45		90		65		65		90	
	AVE.	89	78	-	78	71	78	71	84	48	88	90	89	69	81	95	89	92	78

TABLE 44 (CONTINUED)

SERUM GLUCOSE VALUES EXPRESSED AS mg/100 ml FOR EXPERIMENTALLY INFECTED CATS DURING THE COURSE OF INFECTION

CAT #	SEX	WEEKS POST-INFECTION 20	21	22	23	24	25	27	30	31	35	38
GROUP A												
1	M	65		90		90		80		90		80
2	M		80		90		80		65		65	
5	F		80		90		90		80		90	
6	F		90		90		90		110		90	
7	F	65		110		90		80		80		65
8	F	90		90		80		90				65
	AVE.	73	83	97	90	87	87	83	85	85	82	70
GROUP B												
13	M	80		80		80		65		90		
14	M		80		80		90		90		80	
15	M		80		80		80		65		80	
16	M	90		65		90		65		80		65
18	F		65		65		90		110		65	
19	F		65		80		90		90		65	
20	F	80		80		80		65				90
21	F	90		80		80		65				80
	AVE.	85	73	76	76	83	88	65	89	85	80	78
GROUP C												
22	M		65		90		65		80		90	
23	M	90		60		90		80		90		
24	M	65		65		90		80		65		65
25	M		65		65		90		90		90	
26	F											
27	F		65		80		80					
28	F		65		90		80		90		80	
29	F	65		90		80		80		65		65
	AVE.	73	65	72	81	87	79	80	87	73	87	65

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