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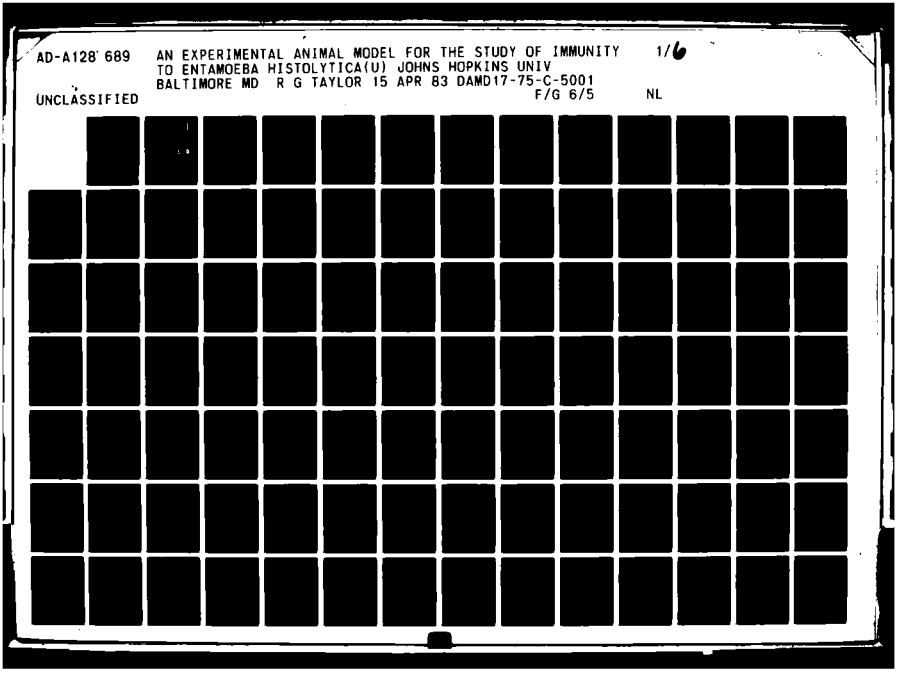

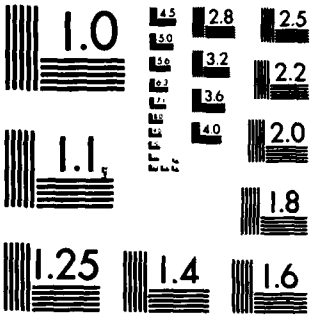

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AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY TO ENTAMOEBA HISTOLYTICA

Final Report

Roy G. Taylor

15 April 1983

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-75-C-5001

The Johns Hopkins University
Baltimore, Maryland 21205

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Comparing experimental hepatic amebiasis in LHC/Lak inbred hamsters with amebic liver abscess as described in man demonstrated the validity of this animal model. An extensive bibliography and review of the literature were given in addition to experimental results. Adult male hamsters were surgically infected via the portal vein with axenically cultivated trophozoites of Entamoeba histolytica. doses of 50 to 315 thousand amebae, strain HM-1:IMSS was the most pathogenic; 200:NIH was intermediate; HU-21:AMC and HK-9 were nonpathogenic. The model exhibited a spectrum of disease depending upon the dose and strain of amebae.

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20 (continued):
Strain 200:NIH provided an opportunity to study healing lesions beginning seven days after infection. Strain HM-1:IMSS was the overall best choice for the model because it caused an infection that mimicked the human disease. Within 48 days after infection, HM-1:IMSS proved lethal to 80% of the animals in a separate group that received the highest dose. Within 15 days this virulent strain caused multifocal caseous necrosis of the liver and hepatomegaly. Gross lesions were solid, and the chronic inflammation involving them was granulomatous with the macrophage being the principal inflammatory cell. Severely infected animals suffered loss of body weight and had a blood picture similar to that of patients with amebic liver abscess. Mild anemia accompanied neutrophilic leukocytosis diminishing with chronicity of the infection. Although total serum protein was normal, the ratio of albumin to globulins was inverted because of decreased albumin and greatly elevated gamma globulin. Appearance of antibodies in the serum coincided with splenomegaly beginning seven days after infection. Bilirubin was normal as in the human disease. Alkaline phosphatase reached extremely high levels in serum and accurately reflected the extent of liver necrosis. Cyclophosphamide, antilymphocyte serum, antithymocyte serum, and neonatal thymectomy in combination with the antisera were used to immunosuppress hamsters before infection with HM-1:IMSS amebae. Antithymocyte serum following neonatal thymectomy exacerbated amebic liver lesions better than the other means of immunosuppression. This result and the known involvement of T cells in granulomatous inflammation suggested that cell-mediated immunity was important in the immunology of experimental hepatic amebiasis.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

INBRED LHC/Lak HAMSTER AS A MODEL FOR AMEBIC LIVER ABSCESS

by

ROY GENE TAYLOR

THESIS

submitted to the School of Hygiene and Public Health
of The Johns Hopkins University in conformity
with the requirements for the degree of

DOCTOR OF SCIENCE

Baltimore, Maryland

1983

ABSTRACT

Comparing experimental hepatic amebiasis in LHC/Lak hamsters with amebic liver abscess as described in man demonstrated the validity of this animal model. Adult male hamsters were surgically infected via the portal vein with axenically cultivated trophozoites of Entamoeba histolytica. At doses of 50 to 315 thousand amebae, strain HM-1:IMSS was the most pathogenic; 200:NIH was intermediate; HU-21:AMC and HK-9 were nonpathogenic. The model exhibited a spectrum of disease depending upon the dose and strain of amebae. Strain 200:NIH provided an opportunity to study healing lesions beginning seven days after infection. Strain HM-1:IMSS was the overall best choice for the model because it caused an infection that mimicked the human disease. Within 48 days after infection, HM-1:IMSS proved lethal to 80% of the animals in a separate group that received the highest dose. Within 15 days this virulent strain caused multifocal caseous necrosis of the liver and hepatomegaly. Gross lesions were solid, and the chronic inflammation involving them was granulomatous with the macrophage being the principal inflammatory cell. Severely infected animals suffered loss of body weight and had a blood picture similar

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Dr. Robert Anderson began as my advisor and strongly supported the desire to do a project of my own design instead of one for which funds were more readily available. Later, Dr. Harvey Fischman accepted the responsibility of being my thesis advisor and stood by me until the work was successfully completed. Both of these men gave generously of their time and sage advice.

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The Division of Medical Audio Visual Services at Walter Reed Army Institute of Research did all the illustrations. Several people ably assisted Paul Klein, Director: George Sangeleer, William Ceresa, Raymond Berry, Dorman Curl, SP4 Phillip Daniel, SP4 Gregory Isom, David Kern, Victor Lagrange, and Donald Stoner.

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Mary Burrow accomplished the difficult task of turning dictation and handwritten manuscript into legible copy. She has been very patient through numerous revisions on the word processor and painstakingly completed this arduous job, which began for her more than two years ago.

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I. INTRODUCTION

The world's literature on amebiasis and Entamoeba histolytica spans more than 1 years. In this chapter, only parts of the literature most pertinent to this study are covered. These topics include an historical synopsis, definition of the term amebiasis, introductory remarks about Entamoeba histolytica, amebic liver abscess in man, effects of immunosuppression on the clinical course of amebiasis in man and animals, and the hamster as a model for amebic liver abscess. The reader may refer to appendix 1 for a more comprehensive review of related subjects.

Objectives

Amebic liver abscess (ALA) is the most important complication of intestinal amebiasis and accounts for considerable morbidity and mortality throughout the world. Yet our knowledge about the pathogenesis and immunology of this disease is severely limited. One cannot hope to find all the answers to questions about pathogenesis and immunology of ALA by studying patients. If all the objections to human experimentation could be satisfied on ethical grounds, other considerations such as cost, small numbers of observations, and lack of uniformity among human subjects weigh

against extensive experimental studies on ALA in man. Of course, epidemiological studies could examine risk factors for amebic liver abscess and hopefully identify population groups at high risk. Then preventive measures might reduce the morbidity and mortality due to this disease. Still, there is a great need to know more about the biology of amebic liver abscess.

The key approach to this problem is a useful animal model for amebic liver abscess to permit meaningful experiments on pathogenesis, pathophysiology, and immunology of the disease. Therefore, the objectives of the present study are to characterize as fully as possible the best available animal model for hepatic amebiasis and to compare the disease in the model with descriptions of the disease in man. No animal model for amebiasis has ever been thoroughly studied in this manner, but the groundwork for this task lies in the accomplishments of others.

History of Amebiasis

For more than one hundred years amebiasis has attracted the best efforts of investigators over the world. The story began to unfold in the nineteenth century with some of the most lucid descriptions and meticulous experiments ever recorded in science or medicine. The fascinating history of amebiasis is the subject of accounts by Scott (1939),

Manson-Bahr (1943), Craig (1944), Anderson et al. (1953), Faust (1954), Hoeppli (1969), Foster (1965), Elsdon-Dew (1968), Martínez Báez (1976), and Imperato (1981). Eight classic papers on amebiasis and Entamoeba histolytica were included in Tropical Medicine and Parasitology: Classic Investigations, which was edited by Kean et al. (1978). In addition, an English translation from Lösch (1875) in which he described "Amoeba coli" was published in 1975 in The American Journal of Tropical Medicine and Hygiene (Lesh [Lösch] 1975). The following is a synopsis on the history of amebiasis and Entamoeba histolytica.

1875 Lösch discovered and described "Amoeba coli" from a Russian farmer suffering from amebic dysentery; he experimentally infected the dog and conducted in vitro experiments on the amebicidal properties of quinine.

1883 Koch was the first person to observe the dysenteric ameba in human lesions of the gut and liver. He did autopsies on five patients who had died of dysentery in Egypt and found amebae not only in histological sections through the bases of intestinal ulcers but also in capillaries of the liver near an abscess (Koch and Gaffky 1887, cited by Dobell 1919).

1886 Kartulis has been credited with attributing the etiology of amebic dysentery to Entamoeba histolytica as he described 150 cases of amebic dysentery in Egypt. The following year he published a paper describing Lösch's ameba in the pus of liver abscesses and implicated the ameba as the cause of amebic liver abscess (Kartulis 1887).

1890 Osler published the first account of amebic dysentery in a patient in the United States.

1891 Councilman and Lafleur first used the terms "amoebic dysentery" and "amoebic liver abscess."[1] They described the course of the disease, made detailed observations on its pathology, and deduced from patients' histories that drinking water was a probable source of infection.

1893 Quincke and Roos described the cysts of Entamoeba histolytica and distinguished between this parasite and Entamoeba coli (Quincke and Roos 1978, translation).

1901 Harris produced experimental amebic liver abscess in puppies.

1903 Schaudinn named the "dysentery amoeba" Entamoeba histolytica (Schaudinn 1978, translation).

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[1]See Harvey (1980).

1904 Musgrave and Clegg coined the term "amebiasis."

1912 Rogers demonstrated the efficacy of hypodermically administering emetine hydrochloride for the treatment of amebiasis.

1913 Walker and Sellards proved by experimental infections in man that Entamoeba histolytica is pathogenic whereas E. coli is not.

1917 Dale and Dobell used experimentally infected kittens for evaluating drugs for the treatment of amebiasis.

1919 Dobell published The Amoebae Living in Man, a scholarly work that made sense out of much prior confusion regarding the identity and pathogenic potential of intestinal protozoans.

1925 Boeck and Drbohlav successfully cultivated Entamoeba histolytica in vitro.

1927 Craig published preliminary work with his complement fixation test for amebiasis, which was described fully two years later (Craig 1929) and became the first successful serologic test specific for antibodies against Entamoeba histolytica.

1928 Dobell described the complete life cycle of Entamoeba histolytica.

1931 Dobell showed that Entamoeba histolytica from monkeys and man is identical.

1938 Faust described the method of zinc sulfate flotation for concentration of amebic cysts in feces (Faust et al. 1938).

1955 Phillips demonstrated a synergistic relationship between bacteria and Entamoeba histolytica, which produced lesions in the cecum of gnotobiotic guinea pigs (Phillips et al. 1955).

1961 Diamond described axenic cultivation of Entamoeba histolytica in a diphasic medium.

Definition of Amebiasis

Musgrave and Clegg (1904) coined the term amebiasis in referring to infection with any ameba whether it is pathogenic or nonpathogenic. Modern usage has restricted the term to mean infection only with Entamoeba histolytica (World Health Organization 1969). Clinical symptoms are not requisite for use of the term.

Entamoeba histolytica Schaudinn 1903

First described by Lösch in 1875, Entamoeba histolytica is the pathogenic intestinal ameba of man. The International Commission on Zoological Nomenclature finally resolved nomenclatural disputes concerning this organism in 1954.

Natural infections occur in other animal species as described later. The habitat of this parasite is the lumen of the large intestine, but it can become tissue invasive and cause ulcers of the colon or even extra-intestinal infection.

In meticulous fashion, Dobell (1928) described the complete life cycle of E. histolytica in culture. Following the trophic stage is the precystic ameba, smaller than the trophozoite, which begins to extrude from its cytoplasm various inclusions. The process of encystation involves the formation of a uninucleate cyst from the precystic ameba by laying down a cyst wall. By successive nuclear divisions the single nucleus finally becomes four in the mature cyst. The nuclei in the cyst stage are similar to those of the trophozoite but somewhat smaller. A glycogen vacuole and chromatoidal bars are usually present in the young cyst but gradually disappear. The process of excystation does not begin until the chromatoidal bars and glycogen are absorbed. The protoplasmic contents escape through a minute perforation in the cyst wall by back-and-forth flowing of the protoplasm. The organism that escapes the cyst wall is an ameba with four nuclei. The quadrinucleate ameba produces eight uninucleate metacystic amebae, which become trophozoites. Swartzwelder (1939) verified these same events

during amebic excystation in the ileum and large intestine of the dog.

Extra-intestinal Amebiasis

Extra-intestinal amebiasis includes involvement of many organs such as liver, lung, brain, spleen, pericardium, urogenital tract, and even the skin.

Classification of Hepatic Amebiasis

Hepatic involvement is by far the most common manifestation of amebiasis outside the intestine (World Health Organization 1969, Wilmot 1962). An expert committee of the World Health Organization (1969) recognized acute nonsuppurative hepatic amebiasis as one form of the disease that is clinically indistinguishable from amebic liver abscess, but no abscess can be demonstrated in the non-suppurative form. Rogers (1922) recognized another form of hepatic amebiasis besides amebic liver abscess that he called "presuppurative amoebic hepatitis." Further discussion of extra-intestinal amebiasis is limited to amebic liver abscess.

Amebic Liver Abscess

Amebic liver abscess is a necrotic lesion caused by Entamoeba histolytica invading the liver. Lesions may be single or multiple from microscopic size to occupying an

entire lobe of the liver. In the absence of secondary bacterial infection, amebic liver abscess is not a true abscess because the contents do not consist of pus cells. A fibrous capsule may or may not be present.

Clinical Manifestations

Clinical manifestations of amebic liver abscess were clearly described by Councilman and Lafleur (1891), who encountered abscesses of the liver in seven of 15 patients with amebic dysentery. Pain and fever were invariably exhibited by their patients. According to Adams and MacLeod (1977b) and Barbour and Juniper (1972), at least three-fourths of patients with ALA have a history of clinical symptoms less than four weeks' duration. In contrast, Ochsner and DeBakey (1943) reported that 62% of their patients had a history of symptoms greater than three weeks' duration. Although almost all patients with ALA have pain, the proportion with concurrent or previous dysentery represents a minority (Ochsner and DeBakey 1943, Adams and MacLeod 1977b) or a small majority (Wilmot 1962, Barbour and Juniper 1972). Sheehy et al. (1968) found that malaise, weight loss, chills, and night sweats were frequently mentioned by their patients. Their paper must be read carefully, however, because they included a group that did not have characteristic pus aspirated from the liver.

Barbour and Juniper (1972) and Ochsner and DeBakey (1943) also mentioned weight loss and chills, whereas nausea and vomiting were less frequent.

By far the most important signs are fever accompanied by an enlarged, tender liver. All of the authors cited above have been consistent on this point. Three-fourths of Wilmot's (1962) patients had an elevated diaphragm, usually on the right side; but other authors have mentioned this finding in less than half of their patients (Adams and MacLeod 1977b, Barbour and Juniper 1972). Jaundice is not a common finding in patients with ALA (Ochsner and DeBakey 1943, Wilmot 1962), but Sheehy et al. (1968) and Barbour and Juniper (1972) reported jaundice in 22% and 30% of their patients, respectively.

Complications

Complications of amebic liver abscess include rupture or extension, bacterial infection, and hematogenous spread to other sites. Ochsner and DeBakey (1943), Sepúlveda et al. (1959), Wilmot (1962), and Adams and MacLeod (1977b) have published thorough reviews of the subject. Working in India, Rogers (1922) attributed many bacterial infections of amebic liver abscess to accidental contamination during open drainage of the lesions. Even modern medical practice does not entirely

avoid secondary infection of ALA during aspiration (Maddison et al. 1959).

Diagnosis

Before the advent of radiology and specific serologic tests for amebiasis, demonstration of Entamoeba histolytica in aspirated pus or in tissue sections was the only means of establishing a definitive diagnosis for amebic liver abscess (Councilman and Lafleur 1891, Rogers 1922). Aspiration and direct microscopic examination to include Gram stain are still valuable in establishing the diagnosis (Wilmot 1962). It is no longer considered necessary, however, to demonstrate the parasite if characteristic amebic pus has been aspirated from a liver abscess, particularly if the abscess is sterile (Craig 1944, Adams and MacLeod 1977b). Councilman and Lafleur (1891) were impressed with finding a great preponderance of abscesses in the right lobe of the liver. Ochsner and DeBakey (1943) offered an explanation for this phenomenon based on a higher prevalence of amebic lesions in the right half of the colon and the particular circulatory pattern of the portal vein draining that part of the intestine. Wilmot (1962) and Adams and MacLeod (1977b) have remarked on the usefulness of radiology to visualize defects in the liver and to confirm an elevated right diaphragm. Arteriography (Lydon and Dodds 1974), ultrasonography (Monroe et

al. 1971, Rasmussen et al. 1973) and scintigraphic scanning of the liver (Gordon et al. 1973, Bieler et al. 1974) are all useful tools in the differential diagnosis of ALA. Serologic tests for amebiasis are highly specific and sensitive for extra-intestinal amebiasis but in rare instances may be nonreactive (Stevens et al. 1979). Antibodies against E. histolytica have been demonstrated in liver aspirate (Mahajan et al. 1975b, Stevens et al. 1979). Mahajan and coworkers (Mahajan et al. 1974a, Mahajan and Ganguly 1980) were able to demonstrate antigen from amebae in pus aspirated from amebic liver abscesses, but this technique using counterimmunoelectrophoresis has not been widely adopted. Barbour and Juniper (1972) and May et al. (1967) have discussed the problems in differentiating amebic and pyogenic abscesses of the liver.

Sometimes a physician does not diagnose amebic liver abscess in time to begin effective treatment, and the consequences can be tragic. Dykes et al. (1980) reported severe amebiasis in three infants, two of whom died after fulminant illness complicated by amebic liver abscess and peritonitis. Entamoeba histolytica had not been seen in stool specimens, and diagnosis was delayed until too late. The infants may have acquired amebiasis from their mothers, who were asymptomatic carriers of the parasite.

Pathophysiology

Pathophysiological measurements are less useful in the diagnosis of ALA than they are in helping to characterize the host response to the infection (Powell 1959b). Powell (1959a, b) published key papers on pathophysiology of this disease and was very careful to compare the findings in amebiasis patients with the findings from patients from the same African population that had other diseases commonly included in the differential diagnosis of ALA. This practice gave him more confidence in attributing abnormalities to the amebic infection. Anemia is a consistent finding in one-half (Wilmot 1962) to two-thirds (Adams and MacLeod 1977b) of the Durban population having amebic liver abscess. In the United States, Barbour and Juniper (1972) listed anemia as an abnormal finding in all 33 of their patients. The anemia in African patients was normocytic becoming hypochromic as the disease progressed and the abscesses increased in size (Mayet and Powell 1964). Rogers (1922) was the first to recognize leukocytosis as a common finding in ALA. Extremely high leukocyte counts are usually associated with multiple abscesses (Barbour and Juniper 1972) or secondary infection of the liver abscess (Ochsner and DeBakey 1943). Ochsner and DeBakey (1943) found the leukocytosis is commonly a neutrophilia, but eosinophilia is not associated with ALA.

Powell (1959b) found that the total serum protein concentration is normal in patients with amebic liver abscess although the A:G ratio is often inverted and less than that of African controls. This observation results from a decrease in albumin accompanied by an increased globulin fraction. Serum electrophoresis reveals increased alpha-1 and alpha-2 globulins, normal beta globulin, and markedly elevated gamma globulin. Santhanagopalan et al. (1964) and Kamat et al. (1968) had similar findings in India.

Liver function tests have also been used to characterize the pathophysiology of amebic liver abscess, but the changes are nonspecific and simply indicate hepatic dysfunction (Powell 1959a). One of the most consistent findings in approximately two-thirds of patients with ALA is elevated serum alkaline phosphatase (Sepúlveda et al. 1959, Salako 1967, Kamat et al. 1968, Barbour and Juniper 1972). Powell (1959b) and Sheehy et al. (1968) found this enzyme to be elevated in 37% and 44% of their patients, respectively. Bilirubin is almost always normal (Wilmot 1962, Powell 1959b). Few authors have studied the transaminases in patients with ALA. Sheehy et al. (1968) and Santhanagopalan et al. (1968) found aspartate aminotransferase was significantly elevated in two-thirds and four-fifths of their

patients, respectively. In contrast, alanine aminotransferase was elevated in only one-third of the patients studied by Santhanagopalan et al. (1968). Two papers reported decreased levels of cholinesterase in the serum of patients with ALA (Magill and Killough 1958, Santhanagopalan et al. 1968).

Pathology and Pathogenesis

The pathologic processes in amebic liver abscess are similar to those of amebic dysentery because the action of Entamoeba histolytica is consistent from one type of tissue to the next (Brandt and Pérez Tamayo 1970). Adequate descriptions of the pathology may be found in several sources (Anderson et al. 1953, Brandt and Pérez Tamayo 1970, Pérez-Tamayo and Brandt 1971, Connor et al. 1976). Detailed observations on the pathology of ALA were made by Councilman and Lafleur (1891), who found the earliest lesions to be 1-5 mm in diameter. The cut surface of such lesions demonstrates their solid nature resembling the caseous necrosis of tubercles caused by Mycobacterium tuberculosis. The largest abscesses may be the size of a child's head (MacNeal and Klemperer 1925), sometimes occupying an entire lobe of the liver (Ochsner and DeBakey 1943). Although many authors have referred to the fluid contents of an amebic liver lesion as pus, amebic liver abscess is really a misnomer

(Councilman and Lafleur 1891) because pus cells indicative of abscess are conspicuously absent unless the lesion has been secondarily infected with bacteria.

The pathogenesis of amebic liver abscess has been debated since Kartulis (1889) postulated that amebae reach the liver by means of the portal vein. Councilman and Lafleur (1891) felt that amebae probably reach the liver most frequently through the abdominal cavity although they did not discount the possibility of infection via blood vessels. Having seen amebae in both blood vessels and lymphatics, they did not believe the lymphatics an important route for metastasis of the intestinal infection because lymphatic glands never contain amebae upon microscopic examination. Although the consensus seems to be that amebae reach the liver via the portal vein (Ochsner and DeBakey 1943), there is experimental evidence that amebae are capable of reaching the liver intraperitoneally in hamsters (Jarumilinta and Maegraith 1962) and by the portal vein in guinea pigs (Maegraith and Harinasuta 1954b).

An amebic lesion begins in the liver as emboli of amebae lodge in small radicles of the portal vein (Rogers 1922). At this early stage, cytolysis of liver tissue is accompanied by little inflammation (Palmer 1938). Portal thrombosis in the liver causes the formation of infarcts,

which appear as wedge-shaped areas containing small amebic abscesses (Palmer 1938). Nearby abscesses coalesce to form larger ones (Palmer 1938), and amebae invade the surrounding tissue producing focal areas of necrosis (Rogers 1922). As the abscess becomes larger, connective tissue walls off the abscess with a fibrous capsule (Councilman and Lafleur 1891, Palmer 1938) eventually resulting in a dense fibrous wall surrounding a large chronic abscess (Councilman and Lafleur 1891, Rogers 1922).

The macroscopic appearance of the smallest abscesses is that of solid white lesions grading into yellow gelatinous ones as they become larger (Faust 1954). Still larger ones may take on a reddish brown appearance due to the presence of blood elements and fibrin in the necrotic center (Faust 1954). The inner surface of the abscess wall frequently has a shaggy appearance due to shreds of partially cytolyzed connective tissue elements; the wall eventually becomes smoother in older chronic abscesses (Craig 1944).

Microscopic examination of the small solid lesions shows them to have an indistinct boundary with the necrotic center being pale and granular. Amebae are numerous in those areas containing degenerating and necrotic liver cells, cell fragments, nuclei, and a few leukocytes (Councilman and Lafleur 1891). Faust (1954) found that

as necrosis becomes extensive, large numbers of polymorphonuclear leukocytes infiltrate the area. As the abscess enlarges, three zones can be recognized in tissue sections: a necrotic center surrounded by a median zone of degenerating cells and an outermost zone of nearly normal tissue being invaded by amebae (Faust 1954). Here and there one may find bits of liver tissue, which has resisted the necrotic process, inside an abscess (Councilman and Lafleur 1891). Bile duct proliferation in the surrounding hepatic parenchyma is more notable in cases with pronounced fibrosis (Palmer 1938). Increased numbers of lymphocytes and mononuclear cells may occur in the portal tracts, and fatty degeneration is a frequent finding (Palmer 1938). Although amebae may be found in the contents of the abscess, they are much more numerous at the periphery (Councilman and Lafleur 1891) and may be found invading normal liver tissue surrounding an abscess (Ochsner and DeBakey 1943). The healing process is a dynamic one involving regeneration of liver cells (Councilman and Lafleur 1891) and formation of connective tissue so that in time all traces of an amebic liver abscess may disappear (Kamat et al. 1970, Brandt and Pérez Tamayo 1970). There is one brief report on the ultrastructure of Entamoeba histolytica in human ALA

(Proctor 1976), but it does not concern the pathology or pathogenesis of this disease.

Treatment

The treatment of amebic liver abscess may very well involve aspirating the abscess to promote healing (Wilmot 1962, Adams and MacLeod 1977b), particularly for larger abscesses (Barbour and Juniper 1972). Opinion is still divided on the need for it, however (Sheehy et al. 1968, Peters et al. 1981). Resolution time for a healed amebic liver abscess as determined by liver scanning varies from two months to more than one year with a median of four months in those patients for whom the diagnosis has been proved by aspiration of characteristic pus (Sheehy et al. 1968). Apparently, the size of the liver abscess and the use of aspiration have little bearing on the speed of healing. Other studies have attempted to answer the question about resolution time, but all of them were flawed by questionable diagnostic criteria for ALA (Tandon and Rajan 1967, Rao et al. 1973, Poulose et al. 1974). Chemotherapy of amebiasis is discussed in appendix 1 under intestinal amebiasis.

Immunology

Today, the immunology of amebiasis remains mostly an enigma. A vaccine for use in man may be available (Sepúlveda 1980b) before basic immune mechanisms in amebiasis are clearly understood. Several authors have reviewed current knowledge about the immunology of amebiasis (Balamuth and Siddiqui 1970; Kagan 1973; Krupp and Jung 1976; Sepúlveda 1976, 1980a; Trissl 1982). The advent of axenic[1] culture methods for Entamoeba histolytica (Diamond 1961, 1968b; Diamond et al. 1978a) and methods for preparing and standardizing antigens harvested from mass axenic cultures (Thompson et al. 1968) heralded the beginning of modern experiments in this area. Beforehand, the contribution of microbial associates to immunological events was an unknown quantity.

Immunosuppression

Some insight to the immunology of amebiasis has been gained from noticing that the disease can be exacerbated in immunocompromised hosts. Administration of steroids to patients for other conditions or because of misdiagnosis has resulted in fulminant amebic dysentery (Kanani and

[1]This terminology and its usage are from Dougherty (1953, 1959).

Knight 1969a, b) or amebic liver abscess (Stuiver and Goud 1978). Unrecognized amebic infection or asymptomatic intestinal amebiasis may presage serious or fatal amebic disease in patients put on steroid treatment (Kanani and Knight 1969b). Bailenger et al. (1972) reviewed earlier cases in the literature. Abioye (1973) has suggested that some type of immunological impairment or hormonal imbalance in pregnant Nigerian women increases their risk of dying from amebic dysentery.

Steroids have been used to increase the severity of intestinal lesions in guinea pigs (Teodorovic et al. 1963) and rats (Kasprzak 1968, Vinayak et al. 1979a) and to make guinea pigs more susceptible to amebic liver abscess (Biagi F. et al. 1963). Whole body irradiation has a similar effect on cecal amebiasis in rats (Vinayak et al. 1979a). By rendering mice tolerant to egg albumen, Lancastre et al. (1968) produced tissue-invasive amebiasis in the cecum. Using the Biswas strain of E. histolytica known to be pathogenic in C3H/mg mice (Neal and Harris 1977), Wijesundera (1980) studied the effects of immunosuppression on cecal infection in this model. Neither cyclophosphamide nor antilymphocyte serum (ALS) alters the outcome. However, liver lesions histologically similar to those in hamsters and man occur in mice immunosuppressed with ALS.

Wijesundera (1980) postulated that T cells may be involved in protecting the mouse against amebic invasion of the liver. This hypothesis could be tested in the athymic nude mouse. Capín et al. (1980a) found that depletion of complement (C) by cobra venom factor exacerbates hepatic amebiasis in hamsters in terms of frequency and severity of lesions. The production of antiamebic antibodies is not altered by C depletion. Interpretation of these results was complicated by the use of monoxenic E. histolytica. Tanimoto-Weki et al. (1974) also used monoxenic amebae in their experiments on immunosuppression in hepatic amebiasis of the hamster. Administration of hydrocortisone or azathioprine to hamsters leads to amebic liver lesions whereas untreated animals given the same dose of amebae are not infected.

Further ablation experiments with axenic amebae in a suitable animal model are indicated to investigate the immunology of amebiasis.

Animal Models

Animals have been used for experimental infection with Entamoeba histolytica since the discovery of the organism by Lösch in 1875. Lösch described "Amoeba coli" from the dysenteric stools of a 24-year-old farmer who had been admitted to clinic in St. Petersberg. The Russian physician not only experimented with various dilutions of quinine

sulfate for killing the amebae but also attempted to infect four dogs experimentally. He gave one or two ounces of fresh feces containing live amebae both orally and rectally to the dogs for three days. One of the dogs developed bloody mucous dysentery and amebae were found in the stools. Autopsy 18 days after infection revealed amebae in the rectal mucus and in ulcers of the rectum (Lesh 1975, translation).

Various models for amebiasis have been reviewed by Anderson et al. (1953), Hoare (1959), and Thompson (1959, 1971). Most animal experiments were chemotherapeutic studies or were designed to study the pathogenicity of Entamoeba histolytica, its pathology, immunology, or host-parasite relationships. Anderson et al. (1953) discussed certain shortcomings of earlier studies. Early workers tended to ignore dissimilarities between their models and the disease in man. Frequently, no pathology was done and the mere presence of amebae was taken as evidence of true infection. Unnatural routes of infection, irritants, and surgical procedures were often employed to establish experimental infections. This criticism is not entirely justified, however. Dogs, monkeys, and rats may be naturally infected with E. histolytica, a situation that could easily confound experimental results. In addition to the deficiencies noted

above, one must add that experimental animals can harbor other members of the genus Entamoeba. Finally, before the advent of axenic cultures the contribution of contaminating microorganisms always clouded interpretation of experiments. Investigators should choose an axenic ameba strain with a documented history and one having predictable pathogenicity in the model.

The extensive literature on animal models for amebiasis is reviewed in appendix 1. Here the discussion is limited to the hamster, the best animal for studies on amebic liver abscess.

Golden Hamster, Mesocritetus auratus

The work of Reinertson and Thompson (1951) was the beginning of many studies using the hamster as a model for amebiasis. Their surgical technique or slight modifications of it have been used by several authors to produce hepatic amebiasis in hamsters (Williams 1959; Jarumilinta 1966; Hernández-López and Escobedo-Salinas 1970; Michel and Westphal 1970; Tanimoto et al. 1971a; Aguirre-García et al. 1972, 1973; Bos 1973; Gold and Norman 1979; Capín et al. 1980a). Amebic liver abscess can occur spontaneously in hamsters that are inoculated intracecally with E. histolytica (Jarumilinta and Maegraith 1961, Ghadirian and Meerovitch 1978a). But a reliable method has not been

devised for inducing amebic liver lesions arising from amebic ulcers in the intestine of hamsters or any other animal model. Therefore, artificial means of infection have necessarily been used to produce hepatic amebiasis in hamsters. Wiles et al. (1963) concentrated amebae from human ALA or used monoxenic amebae and injected them via laparotomy directly into the liver parenchyma. Although most investigators (Raether 1971; Diamond et al. 1973, 1974a; Ghadirian and Meerovitch 1978a, 1979) performed laparotomies before intrahepatic inoculation, Michel and Westphal (1970) found that hamsters can be infected with monoxenic amebae by injecting them through the skin into the liver. This method is also successful with axenic amebae in newborn hamsters (Mattern and Keister 1977b, Martínez-Palomo et al. 1980). Reinertson and Thompson (1951) briefly mentioned success using the portal vein as a route of infection, but few have tried it (Wiles et al. 1963, Wittner and Rosenbaum 1970, Gögler and Knight 1974).

Jarumilinta and Maegraith (1962) confirmed in hamsters the hypothesis of Council'man and Lafleur (1891) that Entamoeba histolytica could infect the liver after reaching the abdominal cavity. Amebae inoculated into the peritoneal cavity with or without laparotomy produce liver lesions and occasionally intestinal lesions in

hamsters, but not in guinea pigs. Later, Jarumilinta (1966) devised a technique for localizing amebae on the surface of the liver by means of a gelatin sponge placed between two lobes of the liver. However, she found no difference in severity of lesions or infectivity of amebae when three methods of infection were compared in hamsters: intrahepatic inoculation by the method of Reinertson and Thompson (1951), intraperitoneal injection via laparotomy (Jarumilinta and Maegraith 1962), and the gelatin sponge method (Jarumilinta 1966). Dutta (1970) exposed just the outer muscle layer of the abdomen before injecting xenic amebae into the peritoneal cavity near the liver. Amebic lesions developed in the liver. Ghadirian and Meerovitch (1978a, 1979) successfully introduced axenic amebae into hamsters by the intraperitoneal route to produce amebic lesions in the liver and elsewhere in the abdominal cavity.

Extra-intestinal amebiasis has been produced experimentally in hamsters by other routes of infection. Depending upon the strain of monoxenic amebae, ALA but no brain abscesses result from injection of the inoculum into the orbital venous plexus (Michel and Westphal 1970). Although Michel and Westphal (1970) had no success by intracerebrally injecting pathogenic amebae into hamsters, Tanimoto et al. (1971b) demonstrated a hamster model for

amebic brain abscess by injecting monoxenic amebae through the corner of the eye into the brain. Occipital and retroauricular routes of infection were unsuccessful (Tanimoto et al. 1971b). Intradermal inoculation of axenic 200:NIH causes localized lesions at the site of injection (Ghadirian and Meerovitch 1979). Chiari et al. (1978) used intramuscular injection of monoxenic amebae to produce abscesses containing motile amebae, which do not disseminate much beyond the site of injection. However, bacteria in the inoculum seem largely responsible for causing tissue destruction. Mattern and Keister (1977b) inoculated axenic HM-1:IMSS amebae into the thorax of hamsters where trophozoites cause extensive caseous necrosis involving the lungs, diaphragm, and pleura.

The hamster model for hepatic amebiasis has been studied in vitro by using methods for tissue culture and organ culture (Chévez et al. 1976d, e, f). Similar experiments have been done using the hamster cecum as target organ for monoxenic amebae (Chévez et al. 1976a, b, c). This approach may help elucidate host-parasite relationships in amebiasis apart from possible confounding variables in the intact host. Of course, using axenic amebae would simplify the host-parasite interaction.

The hamster model for hepatic amebiasis has found a variety of experimental uses, initially for testing drugs against E. histolytica (Reinertson and Thompson 1951, Williams 1959). Studies on the immunology of amebiasis in the hamster are discussed under immunology in appendix 1.

Many workers have investigated the pathogenicity of Entamoeba histolytica and some of its determinants in the hamster. After Phillips et al. (1955) had demonstrated synergism between E. histolytica and bacteria in the cecum of gnotobiotic guinea pigs, Balsam and Shaffer (1958) observed a similar synergistic effect when K-9 amebae in Shaffer-Frye medium and a gram-negative anaerobic streptobacillus were inoculated intrahepatically into hamsters. Experiments by Wittner and Rosenbaum (1970) and Bos (1973) suggested that contact between amebae and nonpathogenic bacteria or simultaneous inoculation of the two are necessary to produce liver lesions in hamsters. Some strains of E. histolytica gradually lose pathogenicity after being placed in monoxenic culture with Crithidia or in axenic culture (Wittner and Rosenbaum 1970, Bos and Hage 1975). Yet reassociation with bacteria restores pathogenicity of amebae in hamster liver.

Equally valid experiments have refuted these findings about pathogenicity. Wiles et al. (1963) produced

bacteriologically sterile amebic liver lesions in two hamsters and concluded that bacteria are unnecessary for experimental hepatic amebiasis. Aguirre-García et al. (1973) reached the same conclusion. Diamond et al. (1973, 1974a) demonstrated conclusively that axenized amebae can produce liver lesions in hamsters, but virulence varies from strain to strain of E. histolytica. Ghadirian and Meerovitch (1978a, 1979) showed that 200:NIH and IP-106 strains kept in axenic culture for 15 and 11 years, respectively, are still pathogenic at extra-intestinal sites in hamsters by various routes of infection. A single passage through hamster liver enhances the pathogenicity of axenic 200:NIH amebae enough to produce cecal lesions in young hamsters whereas unpassaged 200:NIH is not even infective in the cecum. Axenic IP-106 is pathogenic in one-half the animals inoculated intracecally.

Neal and Vincent (1956) and Vincent and Neal (1960) demonstrated that passage through hamster liver enhances the pathogenicity of E. histolytica. A number of authors have used this technique for their own purposes (Williams 1959, Jarumilinta and Maegraith 1961, Jarumilinta 1966, Singh et al. 1971, Lushbaugh et al. 1978b, Ghadirian and Meerovitch 1979, Gold and Norman 1979). The effect was cumulative with multiple passages of axenic amebae in one

study (Lushbaugh et al. 1978b) but not in another (Gold and Norman 1979). Enhancement of ameba pathogenicity has also been accomplished by adding cholesterol (Sharma 1959, Das and Singh 1965, Singh et al. 1971, Bos and van de Griend 1977, Meerovitch and Ghadirian 1978) or epicholesterol (Bos and van de Griend 1977) to culture medium or by feeding cholesterol to experimental animals (Biagi F. et al. 1962, Das and Singh 1965, Singh et al. 1971). Gold and Norman (1979) failed to enhance pathogenicity of axenic HM-1:IMSS with cholesterol. Lushbaugh et al. (1978b) postulated that loss and enhancement of pathogenicity are interrelated such that prolonged axenic cultivation suppresses production or release of a toxin while manipulations that enhance pathogenicity have the opposite effect on amebae. Selection for less pathogenic forms in culture but more pathogenic ones by passage in animals could also explain the results (Bos and Hage 1975, Bos 1976, Lushbaugh et al. 1978b).

Hepatic infection of hamsters has been used as a biological assay for pathogenicity of ameba strains, not only strains originally isolated from patients with invasive amebiasis and extra-intestinal disease (Diamond et al. 1973, 1974a) but also from asymptomatic cyst passers (Tanimoto-Weki et al. 1973a). Mattern and Keister (1977b) showed that various axenic strains of E. histolytica display a similar

ranking of pathogenicity whether the assay is done in the liver of adult hamsters or newborn hamsters or mouse brain.

The hamster model for hepatic amebiasis has not been adequately characterized. Neither has amebic disease in this model been carefully compared to the disease in man.

Nevertheless, some basic information about the model is known. With the extent of liver necrosis as a measure of outcome, there is a positive dose response depending on the number of axenic trophozoites in the inoculum (Diamond et al. 1973, Ghadirian and Meerovitch 1978a, Martínez-Palomo et al. 1980). Prior "sensitization" to E. histolytica is not required to produce amebic liver lesions in hamsters (Wiles et al. 1963). Two inbred hamsters, LSH/Ss Lak and LHC/Lak, are susceptible (Mattern and Keister 1977b; Ghadirian and Meerovitch 1978b, 1979; Hartman et al. 1980), although most experiments have been done with random bred or outbred hamsters. No one has reported any difference in susceptibility among laboratory reared hamsters, perhaps due to a restricted gene pool in the progenitors for Mesocricetus auratus used in science (Yerganian 1972). Mattern and Keister (1977b) showed that newborn hamsters, whether inbred LSH/Ss Lak or outbred Lak/LVG, are equally susceptible to hepatic infection. Diamond et al. (1974b) observed no sex difference in severity of lesions. Although susceptibility

decreases with age past one day up to three weeks (Mattern and Keister 1977b), Wiles et al. (1963) did not observe any change in susceptibility from one month to one year of age. Almost all experiments have been terminated in two or three weeks, often sooner; but Lushbaugh et al. (1980b) made observations as long as eight weeks after infection.

Pathophysiological changes in the hamster model have been studied very little. Severe liver necrosis due to amebae causes loss of body weight (Bos 1973, Ghadirian and Meerovitch 1978a), hepatomegaly (Diamond et al. 1974b, Ghadirian et al. 1980, Lushbaugh et al. 1980b), and splenomegaly in hamsters (Gold et al. 1978, Ghadirian et al. 1980). In a careful study, amebic necrosis of the liver produced elevated serum levels of alanine aminotransferase and aspartate aminotransferase (Raether et al. 1967). Gamma glutamyltransferase was elevated in another study (Resano-Pérez et al. 1980). Guerrero et al. (1980) published an histochemical study on ALA in hamsters.

The pathology of hepatic amebiasis in the hamster has been studied perhaps more than any other aspect of the model. Much of the work was done with xenic cultures of amebae (Williams 1959, Jarumilinta and Maegraith 1961, Wiles et al. 1963, Jarumilinta 1966, Raether et al. 1967) or with monoxenic cultures (Aguirre-García et al. 1972, 1973).

Interpretation of pathological changes is difficult under these conditions because the contribution of bacteria to the pathologic process can not be discounted. Bos and Hage (1975) have shown that carrier strains of amebae with bacteria produce a purulent "K-abscess" with a fibrous wall unlike sterile lesions produced by other types of inocula. Ameba-crithidia cultures are an improvement for the study of pathology (Michel and Westphal 1970, Raether 1971), but Crithidia itself in association with amebae produces a distinctive type of lesion in hamster liver (Bos and Hage 1975).

Therefore, the best experiments for interpretation of pathology utilize axenic amebae. Axenized HK-9 amebae produces inflammatory, granulomatous lesions with some liquefaction of the central portion in six to 10 days (Aguirre-García et al. 1972). This type of granulomatous lesion has not been described in human ALA (Aguirre-García et al. 1972, Bos and Hage 1975); but of course, an ameboma is granulomatous (Doshi 1969, Levine et al. 1971). Also, there have been few opportunities to study early liver lesions in man (Lushbaugh et al. 1980b). Axenic amebae can also cause a nonpurulent necrotic lesion with minimal inflammation that closely resembles ALA in man (Bos and Hage 1975). If allowed to progress, these lesions develop a

fibrous wall and the necrotic center undergoes liquefaction (Bos and Hage 1975, Lushbaugh et al. 1980b). Lushbaugh et al. (1980b) concluded that the early granulomatous response in hamsters evolves from an histiocytic response into a fibrotic one.

Two studies dealt with ultrastructural changes in the liver of infected hamsters. Unfortunately, Treviño-García Manzo et al. (1970) chose not to examine the lesions in situ, and the inoculum included a bacterial associate with amebae. Lowe and Maegraith (1970c) compared normal uninfected liver with infected organs. Degenerating host cells had coarsely granular cytoplasm, mitochondrial damage, and large vacuoles. Kupffer cells and leukocytes in the necrotic area were undamaged.

Hamsters are used infrequently as a model for intestinal amebiasis although this model has promise. Jarumilinta and Maegraith (1961) successfully inoculated xenic amebae into the cecum. The amebic infection is fatal to many of the animals with lesions, and some of them spontaneously develop hepatic amebiasis. According to the literature, nothing else was apparently done with this model until 1976 when Chévez et al. (1976a, b, c) excised the cecum and colon from hamsters and used the whole organ or fragments of it in vitro for experimental infection with

monoxenic amebae. Ray et al. (1979) produced cecal and hepatic amebiasis at the same time in hamsters. Soon after Diamond et al. (1978b) had demonstrated that axenized HM-1:IMSS amebae produce lesions in the cecum of newborn guinea pigs, Ghadirian and Meerovitch (1978a) successfully inoculated adult outbred hamsters intracecally with pathogenic IP-106 from axenic cultures. Amebae metastasize from the gut to the liver and other extra-intestinal sites in some animals. After one passage through hamster liver, axenized 200:NIH amebae are invasive in the ceca of 1- and 3- but not 6-weeks-old inbred hamsters, strain LHC/Lak (Ghadirian and Meerovitch 1979). Metastatic lesions in the diaphragm and mesentery occur in some of the youngest animals. This model should be investigated further.

In summary, the hamster is the model of choice for hepatic amebiasis. For intestinal amebiasis the hamster has not been fairly evaluated, but preliminary results are encouraging.

Choice of Animal Model

Mesocricetus auratus, the golden hamster, is the best animal model for hepatic amebiasis. It meets almost all the criteria for a good model. First, hamsters are susceptible to hepatic infection with Entamoeba histolytica; and organisms in their gut do not interfere with the experimental infection.

They are small enough for lower husbandry costs but large enough for necessary surgical procedures. Although much is known about the basic biology of hamsters (Hoffman et al. 1968), immunology of the hamster has not been studied like that of the mouse. The disease process of hepatic amebiasis in hamsters seems analogous to that in man, and the spectrum of disease in hamsters may depend on the strain of ameba. Finally, inbred strains of hamster are available to facilitate studies on pathogenesis and immunology.

II. MATERIALS AND METHODS

The overall objectives of this study were to evaluate an inbred hamster model for hepatic amebiasis, to compare the pathology and pathophysiology of the disease in the model with amebic liver abscess as described in man, and to explore the effects of immunosuppression on hepatic amebiasis in the model. The main approach entailed infecting hamsters with axenic Entamoeba histolytica via the portal vein followed by sampling of blood and tissues at intervals after infection during necropsy. The host response to amebic liver disease was studied by a variety of measures such as mortality, pathology, pathophysiology, and effects of immunosuppression. In addition to studying cell-mediated immunity (CMI) in the hamster model by means of an ablation experiment, further study of this question involved the use of athymic nude mice.

Experimental Animals[1]

Adult male inbred LHC/Lak hamsters were used for this study and were procured from Charles River Lakeview, Newfield, NJ. This company also supplied neonatally thymectomized LHC/Lak males. Dr. Chao-Kuang Hsu in the Division of Laboratory Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD, provided specific pathogen free (mouse hepatitis virus) homozygous athymic nude (nu/nu) mice with an outbred Swiss mouse background and normal heterozygous (nu/+) littermates. Individual animals were identified by number on metal ear tags.

Materials

Dr. Louis S. Diamond, National Institutes of Health, Bethesda, MD, provided stock cultures of axenized Entamoeba histolytica in TP-S-1 medium (Diamond 1968b). Amebae for experimental infections were cultured axenically in either TP-S-1 or TYI-S-33 medium (Diamond et al. 1978a). Changes in the biological properties of axenized amebae are not known

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[1]Experimentation with animals met the standards in Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 7823, 1978). Humane laboratory animal care was provided at two facilities accredited by the American Association for Accreditation of Laboratory Animal Care: Johns Hopkins Medical Institutions, Baltimore, MD, and Brooke Army Medical Center, Department of Pathology and Laboratory Services, Fort Sam Houston, TX.

to occur when amebae are adapted from growing in TP-S-1 to TYI-S-33 (Diamond et al. 1978a).

Axenized strains of E. histolytica having different degrees of pathogenicity for hamster liver were chosen for this study. Axenic rather than monoxenic or xenic amebae were used to avoid the possible influence on experimental results of bacteria or other microorganisms associated with amebae in culture. Strains with different degrees of pathogenicity were selected to give a broader spectrum of disease in the hamster than possible with a single strain of ameba. Published incidence and severity of liver lesions in experimentally infected hamsters had permitted ranking the four selected ameba strains in decreasing order of pathogenicity: HM-1:IMSS > HU-21:AMC > 200:NIH > HK-9 (Diamond et al. 1974a). Selection of these strains of E. histolytica was based on their known pathogenicity for hamster liver and the relative ease with which they could be cultured axenically. These four strains were originally isolated from patients with symptomatic intestinal amebiasis, and Diamond axenized them later (Diamond et al. 1972).

Diamond (1968b) and Diamond et al. (1972) have recounted the histories of these strains. In 1967 Sepúlveda and de la Torre isolated strain HM-1:IMSS in Mexico City from a rectal ulcer in a patient with amebic dysentery. In 1970 Juniper

isolated strain HU-21:AMC in Little Rock, AR, from a patient with amebic dysentery. The geographic origin of strain 200:NIH is unknown, but Tobie isolated it in 1948 or 1949 from sigmoidoscopic material taken from an American merchant marine with amebic dysentery. In 1951 Frye isolated strain HK-9 from proctoscopic material taken from a Korean prisoner of war. ABRM was the original designation for strain HM-1:IMSS (Diamond et al. 1973), which was axenized independently in Mexico (Kawashima et al. 1973). Henceforth, its designation in this paper will be abbreviated to HM-1, and HU-21 will refer to HU-21:AMC.

Axenic culture media were prepared from commercially available ingredients. For TP-S-1 medium, TP broth base powder, vitamin mixture 107, and bovine serum were obtained from North American Biologicals, Inc., Miami, FL. Ingredients were combined according to Diamond's method (Diamond 1968b), and the complete medium was sterilized by negative pressure ultrafiltration (0.45 μm filter pore size) before dispensing it into sterile screw cap 125 x 16 mm tubes made of borosilicate glass. TP-S-1 and TYI-S-33 culture media contained 15% bovine serum (v/v) for culturing HM-1 and 10% serum for other strains. Ingredients for TYI-S-33 medium were obtained from commercial sources: vitamin solution 107 and bovine serum (North American Biologicals); trypticase

and yeast extract (BBL, Division of Becton, Dickinson & Co., Cockeysville, MD); glucose, sodium chloride, monobasic potassium phosphate, dibasic potassium phosphate, L-cysteine hydrochloride, L-ascorbic acid, vitamin B_{12}, DL-6,8-thioctic acid, and Tween 80 (Sigma Chemical Co., St. Louis, MO); and ferric ammonium citrate (purified brown pearls) from Mallinckrodt Chemical Works, St. Louis, MO. Complete TYI-S-33 was sterilized by positive pressure ultrafiltration (0.45 μm filter pore size) prior to dispensing. As a sterility check, sample tubes of media were incubated 48 h and examined for turbidity. All culture media were used within two weeks of preparation.

Methods

Axenic Cultivation of Entamoeba histolytica

Published methods of *Diamond (1968b) for axenic* cultivation of Entamoeba histolytica were supplemented by his suggested modifications (personal communication). Axenic cultures in either TP-S-1 or TYI-S-33 were subinoculated twice weekly and were incubated at 35.5 ± 0.5°C. For experimental infections, harvesting amebae involved chilling tubes containing 48- to 72-hour cultures in an ice bath for 5 min prior to centrifugation at 1000 x g for 5 min. Culture medium was decanted and replaced with a smaller amount of

fresh medium. One sample of amebae from the tube was counted on a Coulter counter (Coulter Electronics, Inc., Hialeah, FL) while another sample was used to determine viability of the amebae by motility and exclusion of trypan blue (Diamond 1968a). The concentration of amebae was adjusted to give the desired number of viable amebae in 0.5 ml culture medium. Amebae for routine maintenance of cultures or for experimental infections were handled aseptically.

Animal Infections

Experimental infection of hamsters with amebae necessarily involved a surgical procedure. Although spontaneous hepatic amebiasis has occurred in hamsters with invasive amebiasis of the gut (Jarumilinta and Maegraith 1961, Ghadirian and Meerovitch 1978a), a more reliable route of infection was required to assure that a known number of amebae reached the target organ. Inoculation of amebae into the portal vein (Reinertson and Thompson 1951) seemed the most biologically plausible route of infection and permitted statements about pathogenesis after amebae reach the liver. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (7.5 mg per 100 g body weight), and methoxyflurane was used if additional anesthesia was indicated. Anesthetized animals were weighed, shaved with

electric clippers, and restrained on a surgical board. The abdominal skin was cleansed with topical antiseptics, first with a tincture of benzalkonium chloride followed by two applications of a solution of PVP-I (1-vinyl-2-pyrrolidinone polymers, iodine complex). Aseptic technique was used to perform a midline laparotomy and expose the portal vein. The freshly prepared inoculum contained 5×10^4 to 3.15×10^5 viable trophozoites in 0.5 ml total volume with fresh TP-S-1 or TYI-S-33 medium as vehicle. The inoculum was injected toward the liver into the portal vein with a tuberculin syringe and 26 gauge 1/2-inch needle. Control animals received 0.5 ml culture medium. Bleeding after withdrawing the needle was controlled by applying pressure with a sterile gauze sponge to the wall of the portal vein at the wound. The incision was closed in two layers, the first with interrupted sutures of 4-0 mild chromic surgical gut and the skin with metal clips. Nitrofurazone powder was sprinkled on the incision to discourage infection. The animals were caged separately in a warm room until tney recovered from anesthesia.

Mice were infected with 1×10^6 HM-1 trophozoites by intrahepatic inoculation into the liver parenchyma at 4-6 injection sites. The inoculum consisted of amebae in 0.18 ml total volume with TP-S-1 medium as the vehicle. A

tuberculin syringe and 27 gauge 1/2-inch needle were used to inject the inoculum through the alcohol-cleansed skin of animals anesthetized with methoxyflurane. Control animals received 0.18 ml TP-S-1.

Pathology and Pathophysiology

At 3, 7, 11, and 15 days in one experiment and six days after infection in the immunosuppression experiment, bleeding was done by inserting a heparinized capillary tube into the orbital venous plexus of hamsters anesthetized with ether. Prior to necropsy, mice were anesthetized with ether and bled from the brachial vessels (Young and Chambers 1973) nine or 18 days after infection. One capillary tube was used for WBC and RBC blood counts on a Coulter counter. Two more tubes were used for microhematocrit. Thin blood smears were made and stained with Wright-Giemsa for differential counts. Additional blood was drained through a capillary tube into a 75 x 12 mm test tube and allowed to clot. After blood was obtained, the animals were killed by overdose with ether.

Necropsy on the days specified above for hamsters and mice included a description of gross pathology with emphasis on the liver. Body, liver, and spleen weights were recorded along with size of the organs (form appendix 2). The number, size, and distribution of liver lesions were also recorded.

Representative tissues from liver, spleen, kidney, lung, and intestine were fixed in 10% neutral buffered formalin at 4°C. Paraffin sections were cut 4 µm thick and were stained by routine methods (Luna 1968): Mayer's hematoxylin and eosin-phloxine, McManus' periodic acid Schiff (PAS) stain for glycogen, and Giemsa (Armed Forces Institute of Pathology method). Cecal contents were fixed in MF solution (merthiolate-formalin) for intestinal parasites (Sapero and Lawless 1953).

Livers from infected control animals that were not immunosuppressed in the ablation experiment were selected for a brief electron microscopic study of hepatic amebiasis in hamsters. Routine methods were used to process specimens for transmission electron microscopy (Hayat 1970). Before necropsy, small slices were cut from the liver while the animal was under ether anesthesia. The slices were minced with a razor blade into small bits less than 1 mm in largest dimension while immersed in cold fixative. Tissue from necrotic areas was fixed for 1 h at 4°C in 3% glutaraldehyde in 0.1 M phosphate buffer. Fixed tissues were rinsed twice with the buffer and stored in it at 4°C for up to one week before post fixation with 1% osmium tetroxide in 0.1 M phosphate buffer at 4°C. Post fixed tissues were embedded in a mixture of Epon 812 (Ring Chemical Co., Houston, TX) and Araldite 6005 (CIBA Products Corp., Fair Lawn, NJ), and

sections were cut 900 nm thick with a diamond knife. Thin sections were placed on 200 mesh copper grids, stained with uranyl acetate and lead citrate, and examined on an RCA EMU-4 electron microscope at Brooke Army Medical Center, Department of Pathology and Laboratory Services, Fort Sam Houston, TX.

In addition to values for hematology, pathophysiological variables included several chemical determinations on serum (form appendix 3). Serum protein electrophoresis was done on cellulose acetate with reagents and equipment by Helena Laboratories, Beaumont, TX. Biochemical determinations included total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), aldolase (ALD), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH). Enzyme levels and bilirubin were determined on a 400 µl serum sample by the ABA-100 analyzer (Abbott Laboratories, Pasadena, CA).

Serologic tests for amebiasis were indirect hemagglutination (Kagan 1980a) and spectrophotometrically standardized complement fixation (Kent and Fife 1963, Almeida and Fife 1976) performed in microplates. The antigen (lot #T-4601) was prepared from axenically cultivated HK-9 amebae (Parke, Davis, & Co., Detroit, MI).

Immunosuppression

A variety of immunosuppressive agents was used. Cyclophosphamide (CY) was injected intraperitoneally (i.p.) in a dose of 22.5 mg per 100 g body weight 24 h before experimental infection with amebae. Normal rabbit serum (NRS) (control) and antilymphocyte serum (ALS) (lot #15017) prepared in rabbits were purchased from Microbiological Associates, Bethesda, MD. The dosage for antisera and NRS was 1.25 ml per 100 g body weight (0.93-1.55 ml per day per animal). Hamsters received seven daily intraperitoneal injections of NRS, ALS, or antithymocyte serum (ATS) beginning two days before amebic infection and continuing four days thereafter.

Antithymocyte serum was prepared by immunizing New Zealand white rabbits with thymocytes from LHC/Lak hamsters according to the nonadjuvant two-pulse method (James 1973). Rabbits received two intravenous injections of thymocytes 14 days apart, and blood was collected 7-9 days after the second injection. Antiserum from two rabbits was pooled, inactivated at 56°C for 30 min, sterilized by ultrafiltration, and stored at -70°C until use. The ATS had negligible titer against hamster erythrocytes by hemagglutination and was not absorbed. In vitro cytotoxicity against hamster

thymocytes was measured by trypan blue exclusion (James 1973), and the titer was ≥ 1:40 using 50% viability as the endpoint.

Outline of Experiments

Preliminary work involved gaining familiarity with axenic cultures of E. histolytica, practicing surgical technique, and selecting appropriate doses of amebae. Outbred LVG/Lak hamsters were used for these purposes because they were less expensive than inbred hamsters. After 5×10^4 HM-1 trophozoites were found sufficient to cause necrotic liver lesions in outbred hamsters infected via the portal vein, that dose was selected as the lowest infective dose for all four axenic strains of amebae. The lowest dose 5×10^4 and two higher doses of amebae 1.25×10^5 and 3.15×10^5 were spaced evenly on a logarithmic scale to enable statistical testing for trends due to dose in later experimental designs using inbred hamsters. Inbred hamsters were chosen because they are preferred for studies on pathogenesis and immunology (Gill 1980). Other reasons for choosing this model were explained in the Introduction.

Virulence of three axenic ameba strains (HM-1, 200:NIH, HU-21) was evaluated in a separate experiment. Three groups of 10 LHC/Lak hamsters each were infected with 3.15×10^5

trophozoites of one of the ameba strains, and mortality occurring within 60 days was recorded.

The main experimental approach was a study of hepatic amebiasis using inbred LHC/Lak hamsters to permit comparison of the disease in hamsters with amebic liver abscess in man. Strain of Entamoeba histolytica, dose of amebae, and time after infection were three independent variables that were chosen for experimental characterization of the disease. Sixty-four animals were randomly assigned to four groups of 16 each, one group for each strain of axenic amebae. Within each group there were four levels for dose (5×10^4, 1.25×10^5, or 3.15×10^5 amebae and sham-operated controls that received culture medium instead of amebae) and four levels for time (3, 7, 11, and 15 days post infection). Hamsters were killed and necropsied on the days indicated. Host response to amebic infection was evaluated by studying pathology and pathophysiology in addition to the number and size of liver lesions.

Immunosuppression was used to explore the role of immunity in hepatic amebiasis of hamsters. Because HM-1 was the most pathogenic ameba strain used in earlier experiments, 5×10^4 HM-1 trophozoites were used to infect three normal hamsters, three hamsters injected with NRS, and 15 immunosuppressed hamsters (three animals each in the CY,

ALS, and neonatal thymectomy (TX) plus ATS groups; four in the ATS group; and two in the TX plus ALS group). All 21 animals were necropsied six days after amebic infection.

Another experiment using cyclophosphamide for immunosuppression looked at mortality and pathology in 10 hamsters infected with 3.15×10^5 HM-1 amebae. Ten control animals received only amebae. One-half of the animals was necropsied at seven days, and the remainder was necropsied at 15 days.

Athymic nude mice lack functional T cells and therefore provide an opportunity to study cell-mediated immunity of infectious diseases (Armstrong and Walzer 1978). In the last experiment, six nude mice were given 1×10^6 HM-1 trophozoites intrahepatically; and six nude controls similarly were given culture medium. For comparison, 18 heterozygous littermates were divided into three groups of six each: uninfected control, HM-1, and HM-1 plus cyclophosphamide. The last group received CY in a dose of 15.0 mg per 100 g body weight 24 h before infection. One-half of the animals was necropsied at nine days and the remainder, at 18 days. Premature death of an infected heterozygous mouse left two mice in the nu/+ HM-1 group that was necropsied at 18 days.

Experimental Designs and Statistical Methods

Three of the above experiments were designed so that conclusions about the data would be supported by statistical methods of analysis. The type of analysis for each experiment was specified in the design phase, the experiment was conducted, and statistical analysis was done according to plan.

Approach to Design and Analysis

In these experiments, four possible factors were considered capable by themselves of altering the outcome of an experiment. These factors (independent variables) were strain of ameba, time after infection, dose of amebae, and means of immunosuppression. As appropriate, these factors were incorporated into the experimental designs described below.

In general, several hypotheses had been suggested by derangements in pathophysiology that occur in human amebic liver abscess. For example, hamsters with severe hepatic amebiasis were expected to have elevated levels of alkaline phosphatase in serum. Questions about the effects of Entamoeba histolytica on the experimental animal or questions about the effects of immunosuppression in the animal model prompted additional hyp heses.

Level of Significance

A conservative level of significance where $\alpha = .01$ was adopted for testing hypotheses to minimize the probability of rejecting an hypothesis when it was actually true, i.e., a type I error (Snedecor and Cochran 1967). In addition, α was set at .01 to guard against detecting too many significant differences on the basis of chance. In this study the null hypothesis was usually that experimental group means were all equal to the parameter μ and therefore came from the same population. Out of 695 tests of significance, 119 of them were significant at the .01 level, far in excess of the number (7) expected on the basis of chance alone.

A clear distinction should be made between the statistical significance and biological significance of a result. A result that is statistically significant at the 1% level must be explained because such a result is unlikely to have occurred by chance. On the other hand, one cannot ignore a result clearly having biological significance just because $p > .01$. Therefore, biologically significant hypotheses that approach the .01 level of statistical significance will also be discussed. Taking this approach to hypothesis testing should guard against overlooking the biological significance of results while keeping the objectivity of statistical analysis.

Analysis of Variance and Orthogonal Comparisons

One frequently wishes to test for a significant difference between two treatment groups, e.g., control versus low dose or control versus high dose (if dose had two levels in the experiment). When the number of dependent variables is large and treatments have several levels, as in this study, a large number of *t* tests would be necessary to make all the comparisons of interest. But the probability of finding a spurious significant difference becomes great for even a reasonable number (10 or 15) of such comparisons among pairs of means (Snedecor and Cochran 1967). Therefore, Student's *t* test will not do.

Analysis of variance (ANOVA) is a versatile method of analysis that permits multiple comparisons in simple or complex designs. One is usually interested in testing for the significance of main effects, but interactions between main effects may also be important in some designs. Snedecor and Cochran (1967) and Cochran and Cox (1957) discussed analysis of variance, orthogonal comparisons, and multiple comparisons among means.

An efficient way to examine planned comparisons among means is a set of orthogonal comparisons (contrasts).[1] Multiple comparisons may be planned before conducting the experiment (a priori or orthogonal comparisons) or suggested after examining the data (a posteriori). Orthogonal comparisons are especially useful in testing for trends over time or dose levels. For example, in analysis of variance the sum of squares for a main effect may be partitioned into independent sums of squares, one for each contrast, which has a single degree of freedom (d.f.). If a main effect has 3 d.f., then a set of orthogonal contrasts may represent linear, quadratic, and cubic trends.

Computer Data Processing

Data processing followed a sequence from making observations, to recording data on punched cards, to doing statistical analysis. Regardless of the experiment, a completed form (appendix 3) comprised the original record for experimental observations. Raw numbers were entered onto punched cards and verified. A card reader processed the

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[1] A comparison among treatment totals (or means) may be represented by $L = \lambda_1 T_1 + \cdots + \lambda_i T_i$ where $\Sigma\lambda_i = 0$ with λ_i being a coefficient assigned to its respective treatment total. If $\lambda_i=0$, its T_i does not enter into the comparison. A set of comparisons is orthogonal if the algebraic sum of cross products of the coefficients is zero (Snedecor and Cochran 1967).

cards to create data files in an IBM 4331 computer at the Academic Data Processing Center, The Johns Hopkins University, School of Hygiene and Public Health. Release 9 of the Statistical Package for the Social Sciences (SPSS) (Nie et al. 1975, Hull and Nie 1981) offered appropriate programs for analysis of the data. Parts of REPORT, CROSSTABS, ONEWAY, ANOVA, and MANOVA routines were used from SPSS. Graphs were drawn by using EZPLOT, a computer graphics program available at the University Computing Center, The Johns Hopkins University.

The foregoing general discussion about computer data processing is preface to describing three experimental designs in this study and their analysis. The discussion will proceed from the simplest design to the most complex one.

Immunosuppression Experiment with Hamsters

The experiment on effects of immunosuppression in the hamster model for hepatic amebiasis is a completely randomized design. The one-way layout consists of seven treatment groups with unequal numbers of observations per group. The mathematical model for this design may be represented by this equation:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

where Y_{ij} is an observation in the ith treatment group, μ is the overall mean, T_i is the ith treatment effect, and ε_{ij} is experimental error (Snedecor and Cochran 1967). Treatment effects are fixed. Table 1 is a model table for the analysis of variance in this experiment. Table 2 gives the coefficients for orthogonal contrasts in this experiment.

Nude Mouse Experiment

The experiment with athymic nude mice is a randomized complete block design with equal numbers per cell. The two-way layout consists of five treatment groups with blocks representing two different times. The following equation is a mathematical model for this design:

$$Y_{ijk} = \mu + T_i + \beta_j + T\beta_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is an observation comprised of μ the overall mean; T_i the ith treatment effect; β_j the jth block effect; $T\beta_{ij}$ the treatment x block interaction; and a random component ε_{ijk}, experimental error. Treatment and block effects are fixed. Table 3 is an example of the analysis of variance. Table 4 gives the coefficients for orthogonal comparisons of the treatment groups.

Strain x Time x Dose Experiment

The most complex experiment in this study is a 4^3 factorial design, i.e., three factors (independent variables) at four levels each. In this experiment with hamsters, strain of ameba, time after infection, and dose of amebae are the three factors. A mathematical model helps explain the various contributions toward a single observation in the experiment:

$$Y_{ijkl} = \mu + S_i + T_j + D_k + ST_{ij} + SD_{ik} + TD_{jk} + STD_{ijk} + \varepsilon_{ijkl},$$

where Y_{ijkl} is any observation comprised of μ the overall mean; S_i strain effect; T_j time effect; D_k dose effect; ST_{ij}, SD_{ik}, and TD_{jk} first order interactions; STD_{ijk} second order interaction; and ε_{ijkl} experimental error.

Factorial experiments of this size ($\underline{n} = 64$) often are not replicated (Cochran and Cox 1957). Then a true estimate of ε_{ijkl} cannot be made. Therefore, the STD_{ijk} second order interaction is assumed negligible and is used to provide an estimate of the error term as recommended by Cochran and Cox (1957). Because the STD_{ijk} interaction mean square is probably greater than σ^2 (if it could be estimated), $\underline{F}$ tests for the fixed effects model will compare all variance estimates from the design with an inflated estimate for σ^2. The resulting tests of significance are likely to be conservative and lead to rejection of the null hypothesis less

often. In other words, one may fail to reject some null hypotheses although they are false (type II error).

Cochran and Cox (1957) discussed the advantages of factorial experiments, which were important for this study. Factorial experiments are suitable when it is desirable to examine the effects of several factors at different levels, e.g., strain, time, and dose. If an optimum combination of factors at specified levels is unknown, testing all combinations should include an optimum combination that will produce the desired result. Interactions will elucidate what is happening at various combinations of factors with levels. The disadvantage of a factorial experiment is the size and complexity of it. Investigators may not replicate a factorial design to avoid an experiment of unmanageable size and to conserve resources. Table 5 shows an hypothetical analysis of variance without orthogonal contrasts.

Each of the sums of squares for main effects may be partitioned into three independent sums of squares representing orthogonal contrasts, each having a single degree of freedom. The contrasts for each main effect are given in table 6.

TABLE 1

ANALYSIS OF VARIANCE IN THE IMMUNOSUPPRESSION EXPERIMENT WITH HAMSTERS

Source	d.f.*	SS*	MS*	$\underline{F}$*
Treatment	6	SS_T	MS_T	MS_T/MS_E
(1)Control vs immunosuppressed	1	SS_1	MS_1	MS_1/MS_E
(2)HM-1 vs NRS	1	SS_2	MS_2	MS_2/MS_E
(3)CY vs other immunosuppressed	1	SS_3	MS_3	MS_3/MS_E
(4)ALS vs ATS	1	SS_4	MS_4	MS_4/MS_E
(5)ALS + ATS vs TX	1	SS_5	MS_5	MS_5/MS_E
(6)ALS + ATS vs TX interaction	1	SS_6	MS_6	MS_6/MS_E
Error	14	SS_E	MS_E	

*d.f. = degrees of freedom; SS = sum of squares; MS = mean square; $\underline{F}$ = variance ratio.

TABLE 2

ORTHOGONAL CONTRASTS* FOR THE IMMUNOSUPPRESSION EXPERIMENT WITH HAMSTERS

Contrast	Treatment						
	HM-1	NRS	CY	ALS	ATS	TX+ALS	TX+ATS
(1) Control vs immunosuppressed	+5	+5	-2	-2	-2	-2	-2
(2) HM-1 vs NRS	-1	+1	0	0	0	0	0
(3) CY vs other immunosuppressed	0	0	+4	-1	-1	-1	-1
(4) ALS vs ATS	0	0	0	+1	-1	+1	-1
(5) ALS + ATS vs TX	0	0	0	+1	+1	-1	-1
(6) ALS + ATS vs TX interaction	0	0	0	+1	-1	-1	+1

*An underlying assumption for orthogonal contrasts is that the number of observations in all treatment groups is equal. Although unequal numbers exist in this experiment, the unadjusted contrasts in this table are orthogonal. Subprogram ONEWAY in SPSS compensates for unequal numbers by weighting the coefficients of each contrast so that the weighted sum of cross products equals zero. Then the adjusted contrasts are orthogonal (Nie et al. 1975).

TABLE 3

ANALYSIS OF VARIANCE IN THE NUDE MOUSE EXPERIMENT

Source	d.f.	SS	MS	F
Treatment	4	SS_T	MS_T	MS_T/MS_E
(1) nu vs het	1	SS_1	MS_1	MS_1/MS_E
(2) nu control vs nu HM-1	1	SS_2	MS_2	MS_2/MS_E
(3) het control vs het HM-1 + het CY HM-1	1	SS_3	MS_3	MS_3/MS_E
(4) het HM-1 vs het CY HM-1	1	SS_4	MS_4	MS_4/MS_E
Block (time)	1	SS_B	MS_B	MS_B/MS_E
Treatment x block	4	SS_{TB}	MS_{TB}	MS_{TB}/MS_E
Error	19	SS_E	MS_E	

TABLE 4

ORTHOGONAL CONTRASTS FOR THE NUDE MOUSE EXPERIMENT

	Treatment				
	nu/nu (athymic)		nu/+ (heterozygous)		
Contrast	Control	HM-1	Control	HM-1	Cy HM-1
(1) nu vs het	+3	+3	-2	-2	-2
(2) nu con vs nu HM-1	+1	-1	0	0	0
(3) het con vs het HM-1 + het CY HM-1	0	0	+2	-1	-1
(4) het HM-1 vs het CY HM-1	0	0	0	+1	-1

TABLE 5

ANALYSIS OF VARIANCE IN THE STRAIN x TIME x DOSE EXPERIMENT

Source	d.f.	SS	MS	F
Strain	3	SS_S	MS_S	MS_S/MS_E
Time	3	SS_T	MS_T	MS_T/MS_E
Dose	3	SS_D	MS_D	MS_D/MS_E
Strain x time	9	SS_{ST}	MS_{ST}	MS_{ST}/MS_E
Strain x dose	9	SS_{SD}	MS_{SD}	MS_{SD}/MS_E
Time x dose	9	SS_{TD}	MS_{TD}	MS_{TD}/MS_E
Error	27	SS_E	MS_E	

TABLE 6

ORTHOGONAL CONTRASTS FOR THE STRAIN x TIME x DOSE EXPERIMENT

Main effect	Levels			
Strain	HM-1	HU-21	200:NIH	HK-9
(1) HM-1 vs others	+3	-1	-1	-1
(2) HK-9 vs HU-21 + 200:NIH	0	+1	+1	-2
(3) HU-21 vs 200:NIH	0	-1	+1	0
Time	3 days	7 days	11 days	15 days
(1) Linear	-3	-1	+1	+3
(2) Quadratic	+1	-1	-1	+1
(3) Cubic	-1	+3	-3	+1
Dose	Control	50*	125	315
(1) Control vs infected	+3	-1	-1	-1
(2) Linear	0	-1	0	+1
(3) Quadratic	0	+1	-2	+1

*Dose in thousands of amebae.

III. RESULTS

The primary objective of this study was to demonstrate the usefulness of adult male LHC/Lak hamsters as a model for amebic liver abscess. Studying a range of outcome variables including mortality, pathology, pathophysiology, and effects of immunosuppression facilitated comparison of hepatic amebiasis in the animal model with published descriptions of ALA in man.

If the hamster proves a good model for amebic liver abscess, then similar pathological changes should occur in both man and the model. Knowledge about the human disease leads one to expect hepatomegaly accompanied by necrotic lesions in the liver of infected hamsters. As human lesions become more advanced and larger, they often undergo liquefaction in the center surrounded by inflammation and degenerating liver cells interspersed with amebae. Fibrosis often surrounds chronic lesions. Histopathology should be similar in hamster liver. Infected animals should become anemic with a leukocytosis. Likewise, one may expect an inverted A:G ratio due to increased gamma globulin and decreased albumin in hamster serum. Judging from the human disease, one may predict normal bilirubin but elevated serum

alkaline phosphatase in the model. These pathological changes are most prominent and consistent in the human disease and therefore merit special attention in experimentally infected hamsters. Because immunocompromised patients can experience exacerbation of amebic infection, immunosuppression of hamsters should have a similar result.

Results of the following experiments evidenced the usefulness of LHC/Lak hamsters as a model for amebic liver abscess.

Mortality Experiment

Mortality of infected animals was the simplest way to compare the virulence of axenically cultivated strains of amebae in the hamster model. Here the term virulence refers to the ability of Entamoeba histolytica to overcome host defenses and ultimately cause death of an experimentally infected animal. Pathogenicity refers to the capability of causing disease, and in particular, one or more macroscopic lesions in the liver of an experimental animal. Pathogenicity and virulence are not used interchangeably, but a distinction is made as by Hoare (1952).

Three groups with 10 hamsters in each were infected via the portal vein with 3.15×10^5 trophozoites of HM-1, 200:NIH, and HU-21, respectively. This was the highest dose planned for a larger experiment using hamsters to study the

AD-A128 689 AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY TO ENTAMOEBA HISTOLYTICA(U) JOHNS HOPKINS UNIV BALTIMORE MD R G TAYLOR 15 APR 83 DAMD17-75-C-5001 2/6

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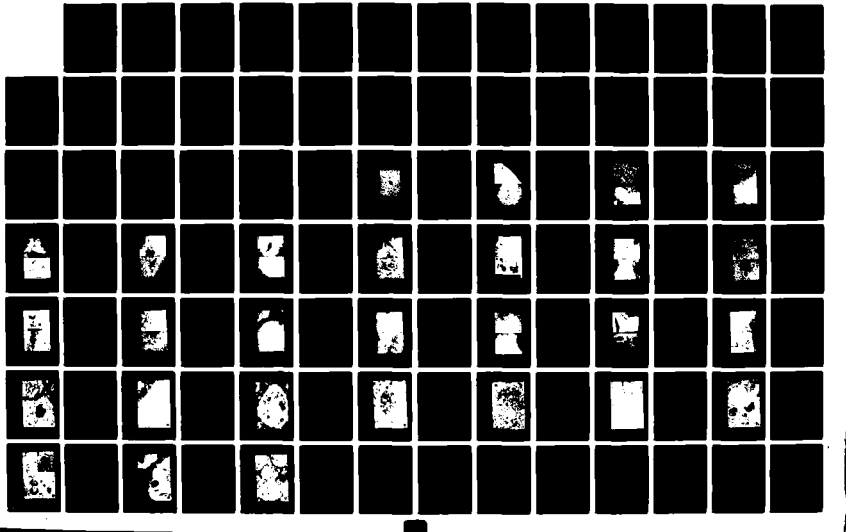
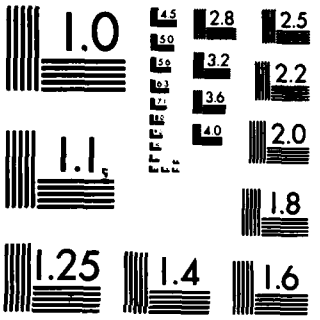




pathology and pathophysiology of hepatic amebiasis caused by the same strains of amebae. The animals were observed for 60 days and mortality was recorded.

Eight of 10 hamsters infected with HM-1 died, but no deaths occurred in the other two groups during the test period. Deaths occurred between four and 48 days. Although the following experiment showed that 200:NIH was capable of producing necrotic lesions in the liver of hamsters, HM-1 was clearly the most virulent strain used in this experiment. The virulence of HM-1 at the dose used here made it inadvisable to exceed 315 000 HM-1 amebae in subsequent experiments using hamsters.

Strain x Time x Dose Experiment

This experiment was designed as a general study of hepatic amebiasis in the inbred hamster model. The overall objectives were to characterize the disease in the animal model and to compare it with the same disease in man. Four different axenic strains of Entamoeba histolytica (HM-1, HU-21, 200:NIH, HK-9) were used at three different dose levels (5×10^4, 1.25×10^5, 3.15×10^5), and the results from infected animals were compared with uninfected but sham-operated controls. The idea was to study the spectrum of disease in hamsters by selecting strains of amebae that exhibited different levels of pathogenicity and by using

various dose levels in the experiment. The best combination of strain and dose could be selected to produce the desired severity of disease in subsequent experiments. Animals were autopsied at 4-day intervals between three and 15 days after infection. Tables 55-60 in appendix 4 give all the experimental data from this experiment.

General Pathology

Number of Lesions

One of the simplest means of assessing hepatic amebiasis in the animals was to count the number of lesions in the liver. Table 7 gives the mean lesion counts for each strain of ameba at four different time intervals. Strains HM-1 and 200:NIH produced more lesions on the average than HU-21 or HK-9, but the difference was not statistically significant (table 8). Uninfected control animals by definition did not have lesions and therefore were excluded from this analysis. HM-1 alone displayed a steady decline in the number of liver lesions over time (table 7). The experimental effects of time and dose on the number of lesions were not statistically significant.

Size of Lesions

The largest lesion in the liver of each animal was measured to compare differences among the strains of amebae.

Similar results obtained whether one compared the greatest or smallest dimensions of the individual lesions; only data for the greatest dimension are presented here. Table 9 presents the analysis of variance for this variable. Before discussing the main effects, the strain x time interaction bears discussion although it was not statistically significant. Figure 1 illustrates that an interaction between strain and time was at play. Strains HM-1 and 200:NIH produced significantly larger lesions than HU-21 or HK-9 (table 9). The time effect had a significant linear component. Although the effect of dose (table 9) was not significant, figure 2 illustrates very nicely that HM-1 produced larger lesions than HU-21 or HK-9. Although the trend was not statistically significant, both HM-1 and 200:NIH seemed to produce larger lesions at higher dose levels (figure 2).

Hepatomegaly

Hepatomegaly was another variable studied for its relationship with the severity of amebic liver disease in hamsters. Table 10 presents mean liver weights for controls and hamsters infected with the four strains of Entamoeba histolytica at various time intervals.

Unlike the other strains, HM-1 showed a consistent enlargement of the liver, which became more pronounced with

time. The overall experimental effect of time had a significant linear component (table 11). The highly significant difference among strains (table 11) was due to HM-1 causing much greater hepatomegaly than the other strains. The overall dose effect was not significant, and control animals did not differ significantly from those that were infected. Nevertheless, figure 3 shows dramatically that HM-1 caused considerable hepatomegaly in animals compared to uninfected animals. Strain 200:NIH caused hepatomegaly only at the highest dose level used. The other strains did not cause hepatomegaly.

Splenomegaly

The spleen is another organ that can respond to infection by enlarging. Table 12 presents mean spleen weights for the four strains of amebae at intervals after infection. The same pattern that occurred with hepatomegaly was evident in these data. Animals infected with HM-1 had larger spleens than controls and animals infected with the other strains with the exception of 200:NIH at 11 days. Otherwise, there was no strain x time interaction except with respect to HM-1. Not only a strain x time interaction but also a strain x dose interaction were evident in the ANOVA (table 13). The latter interaction is graphically represented in figure 4. Both HM-1 and 200:NIH caused

splenomegaly but 200:NIH only at higher dose levels. Neither HU-21 nor HK-9 caused splenomegaly. The highly significant strain effect (table 13) was due primarily to the much larger spleens in animals infected with HM-1. Time did not have an overall significant effect on splenomegaly. The dose effect was highly significant (table 13), and control animals had much smaller spleens than infected ones. Figure 4 shows that a dose response to amebic infection was evident only in spleen weights for animals given HM-1 or 200:NIH.

Summary of General Pathology

The general pathology of hepatic amebiasis in the hamster model mimicked the human disease best when HM-1 amebae were used. Strains HU-21 and HK-9 were essentially nonpathogenic whereas 200:NIH caused pathological changes of intermediate severity in hamsters. As nearby amebic abscesses coalesce to form larger ones in man (Palmer 1938), the number of lesions presumably becomes smaller. This phenomenon was readily apparent in animals that received HM-1. With the passage of time small HM-1 lesions coalesced to form fewer large ones. The data suggested a dose response with respect to size of lesions, but the trend was not statistically significant. An enlarged, tender liver is

a consistent finding in ALA patients (Ochsner and DeBakey 1943, Wilmot 1962, Adams and MacLeod 1977b). In hamsters hepatomegaly was dramatic and became more pronounced with time or larger doses of amebae, but only in the HM-1 group. Splenomegaly associated with ALA has not been emphasized in the medical literature but was remarkable in the animal model, especially in animals given HM-1. Enlargement of the spleen occurred by seven days after infection, and the degree of enlargement was dose related. Splenomegaly in the 200:NIH group was delayed until 11 days, but the same group at 15 days did not have splenomegaly. This aberrant finding with 200:NIH was consistent at 15 days whether the variable of interest was the number or size of liver lesions, liver weight, or spleen weight.

TABLE 7

MEAN LESION COUNTS IN LIVERS OF HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER THREE DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INOCULATION

Time after Inoculation	Strain			
	HM-1	HU-21	200:NIH	HK-9
3 days	21.0	2.7	17.3	3.0*
7 days	13.0	13.3	1.0	.3
11 days	8.0	.3	21.7	4.7
15 days	4.7	.3	.3	1.0
Mean	11.67	4.17	10.08	2.18

* This mean excludes animal 29 with 142 lesions, an obvious outlier.

TABLE 8

ANALYSIS OF VARIANCE IN COUNTS OF LIVER LESIONS AT DIFFERENT LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain(S)	726.34768	3	242.11589	2.13661	.133
HM-1 vs other strains	331.99595	1	331.99595	2.92978	.105
HK-9 vs HU-21 + 200:NIH	184.31006	1	184.31006	1.62649	.219
200:NIH vs HU-21	210.04167	1	210.04167	1.85356	.191
Time (T)	588.85384	3	196.28461	1.73216	.198
Linear	432.56646	1	432.56646	3.81729	.067
Quadratic	22.30656	1	22.30656	.19685	.663
Cubic	133.98083	1	133.98083	1.18235	.292
Dose (D)*	61.90805	2	30.95403	.27316	.764
Linear	9.03125	1	9.03125	.07970	.781
Quadratic	52.87680	1	52.87680	.46662	.504
S x T interaction	1352.36683	9	150.26298	1.32603	.294
S x D interaction	273.21597	6	45.53600	.40184	.868
T x D interaction	184.14005	6	30.69001	.27083	.943
Error	1926.40162	17	113.31774		

* Dose level 0 (control) was excluded.

TABLE 9

ANALYSIS OF VARIANCE IN THE SIZE* OF LARGEST LIVER LESIONS AT DIFFERENT LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

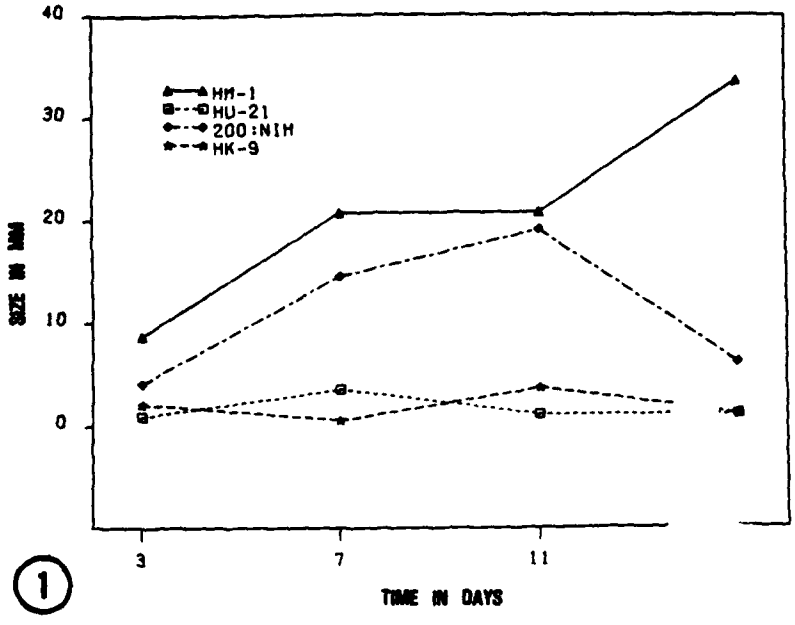
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	1894.93097	3	631.64366	33.50636	.003
HM-1 vs other strains	1403.92970	1	1404.92970	74.47327	.001
HK-9 vs HU-21 + 200:NIH	146.43228	1	146.43228	7.76769	.049
200:NIH vs HU-21	344.56900	1	344.56900	18.27811	.013
Time (T)	487.47079	3	162.49026	8.61951	.032
Linear	415.83734	1	415.83754	22.05864	.009
Quadratic	63.78746	1	63.78746	3.38369	.140
Cubic	7.84578	1	7.84578	.41619	.554
Dose (D)**	133.41863	2	66.70932	3.53868	.130
Linear	109.34271	1	109.34271	5.80023	.074
Quadratic	24.07592	1	24.07592	1.27714	.322
S x T interaction	748.09667	9	83.12185	4.40931	.083
S x D interaction	83.35097	6	13.89183	.73691	.649
T x D interaction	168.77583	5	33.75517	1.79059	.296
Error	75.40583	4	18.85146		

* Size in terms of the largest dimension for each lesion.

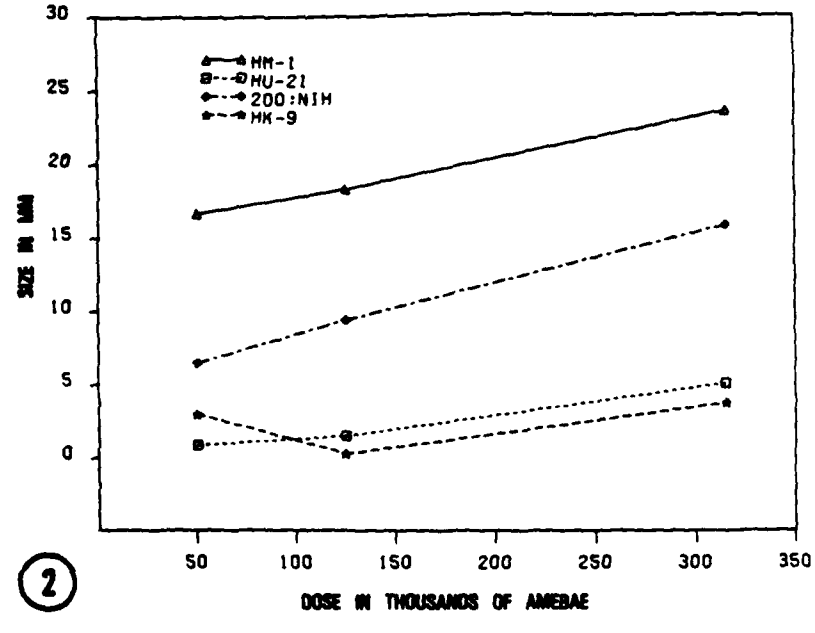
** Dose level 0 (control) was excluded.

Figure 1. Temporal changes in the size of largest amebic liver lesions. Only the most pathogenic strain, HM-1, produced lesions that became consistently larger with time.

Figure 2. Relationship of lesion size to dose of amebae. The size of largest liver lesions was dose related for strains HM-1 and 200:NIH, which produced lesions intermediate in size between HM-1 and the other two strains.


40
30
20
10
0
SIZE IN MM
HM-1
HU-21
200:NIH
HK-9
3
7
11
TIME IN DAYS

1


30
25
20
15
10
5
0
SIZE IN MM
HM-1
HU-21
200:NIH
HK-9
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

2

TABLE 10

MEAN LIVER WEIGHT IN GRAMS FOR HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSIES AT INTERVALS AFTER INOCULATION

Time after inoculation	Strain HM-1	HU-21	200:NIH	HK-9	Uninfected controls
3 days	4.070	3.946	3.593	3.403	4.765
7 days	8.700	3.507	4.810	4.230	4.200
11 days	8.630	3.670	9.273	4.300	4.958
15 days	10.990	4.387	4.393	4.420	4.850
Mean	8.0975	4.2200	5.5175	4.0883	4.6931

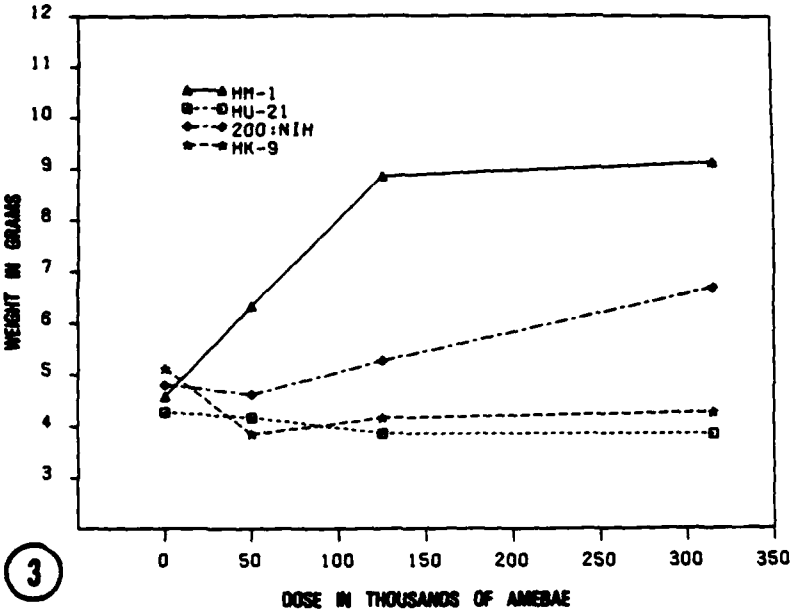
TABLE 11

ANALYSIS OF VARIANCE IN LIVER WEIGHT AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

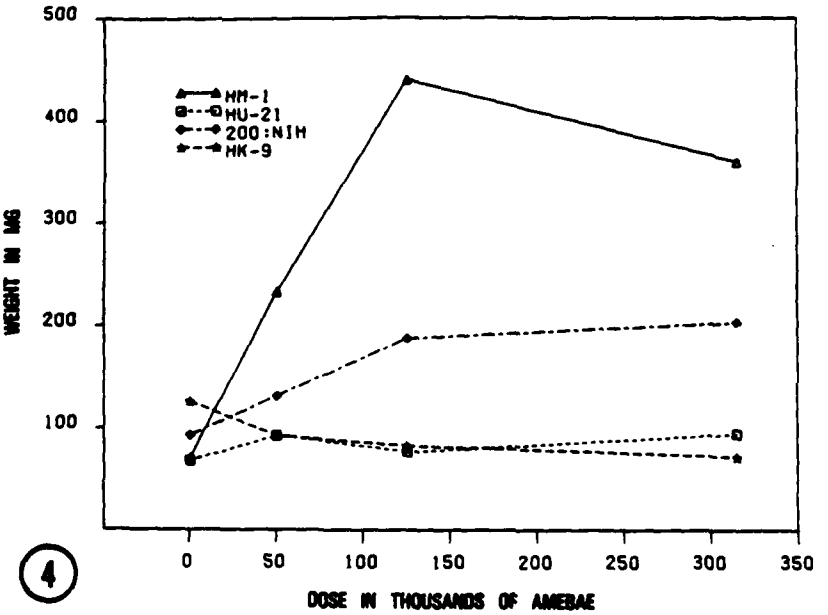
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	99.07903	3	33.02634	10.46664	<.0005
HM-1 vs other strains	84.16403	1	84.16403	26.67308	<.0005
HK-9 vs HU-21 + 200:NIH	1.19930	1	1.19930	.38008	.543
200:NIH vs HU-21	13.71570	1	13.71570	4.34675	.047
Time (T)	47.37229	3	15.79076	5.00437	.007
Linear	34.21728	1	34.21728	10.84407	.003
Quadratic	10.87351	1	10.87351	3.44601	.074
Cubic	2.28150	1	2.28150	.72305	.403
Dose (D)	17.74991	3	6.24997	1.98073	.141
Control vs infected	6.20641	1	6.20641	1.96692	.172
Linear	12.18945	1	12.18945	3.86305	.060
Quadratic	.35405	1	.35405	.11221	.740
S x T interaction	56.90782	9	6.32309	2.00390	.079
S x D interaction	51.80895	9	5.75655	1.82435	.110
T x D interaction	40.11260	9	4.45696	1.41249	.232
Error	85.19557	27	3.15539		

Figure 3. Hepatomegaly related to the dose of amebae. Strain HM-1 caused hepatomegaly at all dose levels whereas 200:NIH did this only at the highest dose.

Figure 4. Splenomegaly as a function of inoculum size. Strains HM-1 and 200:NIH caused splenomegaly, but 200:NIH had this effect only at higher dose levels.


12
11
10
9
8
7
6
5
4
3
HM-1
HU-21
200:NIH
HK-9
WEIGHT IN GRAMS
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

3


500
400
300
200
100
HM-1
HU-21
200:NIH
HK-9
WEIGHT IN MG
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

4

TABLE 12

MEAN SPLEEN WEIGHT IN MILLIGRAMS FOR HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INOCULATION

Time after inoculation	Strain				Uninfected Controls
	HM-1	HU-21	200:NIH	HK-9	
3 days	131.80	114.00	97.33	80.75	123.00
7 days	480.47	88.73	172.53	94.20	84.80
11 days	359.87	79.60	356.27	86.93	80.50
15 days	403.27	67.67	67.93	65.80	69.25
Mean	343.850	87.500	173.517	81.750	89.388

TABLE 13

ANALYSIS OF VARIANCE IN SPLEEN WEIGHT AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	377186.71	3	125928.90	15.55211	<.0005
HM-1 vs other strains	330937.54	1	330937.54	40.87050	<.0005
HK-9 vs HU-21 + 200:NIH	6790.56	1	6790.56	.83863	.368
200:NIH vs HU-21	40058.61	1	40058.61	4.94720	.035
Time (T)	64158.30	3	21386.10	2.64117	.070
Linear	3858.64	1	3858.64	.47654	.496
Quadratic	60294.76	1	60294.76	7.44635	.011
Cubic	4.90	1	4.90	.00061	.981
Dose (D)	111453.60	3	37151.20	4.58814	.010
Control vs infected	81213.61	1	81213.61	10.02981	.004
Linear	15824.17	1	15824.17	1.95427	.174
Quadratic	14415.81	1	14415.81	1.78034	.193
S x T interaction	201096.22	9	22344.02	2.75947	.020
S x D interaction	237915.38	9	26435.04	3.26471	.008
T x D interaction	112253.08	9	12472.56	1.54035	.184
Error	218624.99	27	8097.22		

Histopathology

Axenic Strain HM-1

Hamsters infected with HM-1 amebae provided the best material for studying the histopathology of hepatic amebiasis in this model. Pathological effects of the other three amebic strains will be discussed later although they were less pathogenic than HM-1, which caused reproducible, progressive pathological changes in the liver. For comparison with pathological tissues, figure 5 shows normal histology of the liver in LHC/Lak hamsters.

Injection of trophozoites into the portal vein assured that amebae reached the target organ where they acted as emboli in the portal circulation. Having lodged in small branches of the portal vein, HM-1 amebae caused focal necrosis within 24 hours (figure 6).

Gross appearance of the liver was normal at 24 hours except for numerous discrete, pinkish lesions located preferentially beneath the liver capsule. The necrotic lesions were less than 0.5 mm in diameter. Sections through 24-hour lesions (figure 7) revealed hematophagous amebae (figure 8) in the basophilic centers, which contained necrotic debris, pyknotic nuclei and nuclear fragments, sparse polymorphonuclear leukocytes, and rare mononuclear cells. An acidophilic layer of degenerating and necrotic liver cells with

mixed inflammatory cells separated the necrosis from adjacent normal tissue.

By three days multilocular lesions began to coalesce from individual necrotic foci (figure 9). In the resulting multifocal, necrotizing hepatitis, macrophages were the predominant inflammatory cell type in a layer surrounding the necrosis. Dystrophic calcification was already evident. Amebae were concentrated in the macrophage layer among degenerating and necrotic hepatocytes, PMN, and eosinophils. A sparse, incomplete layer of fibroblasts partially walled off the lesion (figure 10); and mitotic figures were seen. Hepatocytes at the periphery of lesions were often compressed, but amebae did not advance beyond the borders of necrotic foci. Periportal inflammation consisted of mild mononuclear infiltration in areas apart from the lesions themselves.

By five days amebic lesions had developed irregular borders and were sometimes associated with hemorrhagic areas. Enlarging necrotic centers showed dystrophic calcification of necrotic debris but few amebae. Figure 11 shows trophozoites congregated in a feeding zone between the necrotic center and adjacent normal tissue. There the amebae fed on necrotic material and host cells including erythrocytes and leukocytes. A proliferative fibrotic

response was evident. Small abscesses containing neutrophils and amebae were occasionally seen, but PMN cells did not survive the encounter. Periportal areas were infiltrated by mononuclear cells, many eosinophils, and a moderate number of PMN. Formation of secondary foci (figure 12) in the vicinity of multilocular lesions was suggested by small lesions similar to day-old ones.

The liver was greatly enlarged by seven days, and up to 50% of it was damaged by caseous necrosis. Hemorrhage was often associated with the lesions but sometimes occurred in areas of the liver otherwise unaffected. Necrotic lesions were large and multifocal (figure 13). Microscopic examination of the lesions revealed extensive necrosis (figure 14) with some calcification of cell debris. Amebae were usually at the periphery where a layer of macrophages was incompletely surrounded by fibroblasts, mononuclear cells, PMN, occasional eosinophils, and a few plasma cells, resulting in a granulomatous reaction (figure 15). Hematogenous spread of amebae was suggested by finding trophozoites inside branches of the portal vein (figure 16). Proliferation of bile ducts in response to injury (figure 17) was seen for the first time seven days after infection. Hepatic sinusoids surrounding the necrosis were engorged with red blood cells. While extramedullary hematopoiesis

in some animals became a prominent feature in the liver (figure 18) and spleen (figure 19), portal tracts were infiltrated by lymphocytes and eosinophils (figure 18).

Figure 20 illustrates severe thrombophlebitis of the portal vein secondary to hepatic amebiasis nine days after infection. The section reveals an area where amebae may have broken out of the portal vein to cause the large necrotic lesion nearby. Inflammation of the vessel wall included both neutrophils and mononuclear cells.

Necrosis encroached on the remainder of the liver so that lesions extended completely through the thickness of some liver lobes by 11 days. Caseous necrosis occupied much of the pale, greatly enlarged organ (figure 21). Liver tissue had the same firm consistency as amebic lesions, which were very nodular and sometimes were surrounded by hemorrhage. Smaller, more recent foci now filled the spaces between older lesions (figure 22), and significantly larger numbers of amebae occurred in all necrotic foci. Histopathology of multilocular lesions had changed little since seven days after infection except for considerably more fibrosis (figure 23), which completely enclosed some lesions. Severe pericholangitis characterized by infiltrating lymphocytes and occasional eosinophils (figure 24) was the prominent pathological feature in the parenchyma

apart from necrotic foci. In other periportal areas the histological picture suggested formation of lymphoid follicles (figure 25) because masses of plasma cells were seen among the lymphocytes, some with mitotic figures. The medial layer of blood vessels in some portal triads was thickened.

Fifteen days after infection some lobes of the enormously enlarged liver were occupied entirely by yellowish nodular lesions (figure 26). Much of the remaining liver was firmer than normal but not as firm as the necrotic lesions. Adhesions between the liver and diaphragm or other viscera resulted from an occasional large amebic lesion breaking through the liver capsule, but the lungs were not colonized by amebae. Figures 27 and 28 illustrate that amebae were vastly more numerous in all necrotic areas. The chronic inflammatory response was a granulomatous reaction with pronounced fibrosis (figure 29). No part of the liver was unaffected because areas distant from the necrosis were the site of extramedullary hematopoiesis and large accumulations of plasma cells. At this stage the parasite had nearly overwhelmed the host's defenses and was at liberty to spread throughout the liver in blood vessels (figure 30).

Other Axenic Strains

Hamsters that received 200:NIH amebae initially developed the same pathological changes caused by strain HM-1 (figures 31-33). Figure 34 illustrates a microabscess sometimes found in the liver of animals given 200:NIH or HM-1. Although the most severe pathological changes in the 200:NIH group were evident at 11 days, granulation tissue as a sign of repair appeared as early as seven days (figure 35). Only remnants of healing lesions could be found 15 days after infection with 200:NIH (figure 36). Consisting primarily of macrophages and other chronic inflammatory cells, healing lesions occurred near the surface or deep in the hepatic parenchyma. Sometimes a few fibroblasts remained at the periphery, but hepatocytes in the healing lesions were rare. Liver parenchyma near sites of repair was normal, and no amebae were seen in the same tissue sections.

The other strains of axenic amebae, HU-21 and HK-9, were essentially nonpathogenic in the liver of hamsters. Very few of the animals that received these amebae showed any pathologic lesions. The worst damage by HK-9 was a tiny focus of hepatocellular degeneration with mononuclear infiltration three days after infection (figure 37). No amebae were seen in sections. Seven days after infection

with HU-21, the liver of one in 16 hamsters had both active (figure 38) and healing amebic lesions (figure 39); but most of the organ was normal.

Summary of Histopathology

The histopathology of experimental hepatic amebiasis in the hamster was very similar in many respects to amebic liver abscess in man. Axenic strain HM-1 of E. histolytica produced pathological changes closely resembling the human lesions while 200:NIH, a less pathogenic strain, provided an opportunity to study repair of amebic infection in the liver.

The pathogenesis of hepatic amebiasis in the animal model began as axenic amebae entered the portal circulation and lodged as emboli in small branches of the portal vein. Portal thrombosis provided a foothold for amebic invasion of the liver parenchyma, a process like the classic descriptions of Rogers (1922) and Palmer (1938). Focal necrosis in hamster liver was initiated within 24 hours when lesions less than 1 mm consisted of a necrotic center containing trophozoites, surrounded by a layer of degenerating and necrotic liver cells. A necrotizing hepatitis reminiscent of early human lesions (Palmer 1938) was characterized by polymorphonuclear leukocytes and mononuclear cells.

Within three days multilocular lesions began to coalesce from individual small ones. Palmer (1938) believed that large amebic lesions arise by this fashion in the liver of man. At this early stage the predominant inflammatory cell was the macrophage assisted by PMN and eosinophils. Mast cells were conspicuously absent from amebic lesions in hamster liver but did occur normally in connective tissue of the liver. As fibroblasts began to wall off the injury, areas of the liver distant from amebic lesions developed periportal inflammation with mononuclear cells and eosinophils.

Between three and seven days after experimental infection, the gross and microscopic appearance of the liver became worse. Hepatomegaly was pronounced, and caseous necrosis occupied up to 50% of the organ. Large nodular lesions consisted of a necrotic center with some dystrophic calcification of cell debris, a peripheral layer consisting mostly of macrophages and degenerating cells where most of the amebae were found, and an outer layer of fibroblasts next to normal tissue. Similarly, Faust (1954) described three distinct zones in tissue sections of ALA: necrotic center, median zone of degenerating cells, and outermost zone of nearly normal tissue. Extramedullary hematopoiesis and proliferation of bile ducts in response to injury were noted in the animal model. Palmer (1938) found bile duct

proliferation more notable when fibrosis in ALA was pronounced. Pathogenic amebae were able to spread to adjacent areas by direct extension or by using the convenient hematogenous route for establishing secondary foci in hamsters.

The number of amebae in tissue sections increased dramatically after the first week. As in the human disease, trophozoites were more numerous near the edge of lesions (Councilman and Lafleur 1891) but could be found invading adjacent normal tissue (Ochsner and DeBakey 1943). The hamster liver became very reactive and increased to twice or more its normal size. A fibrotic response attempted but failed to contain the necrotic process, which was surrounded by a granulomatous reaction. In contrast, large chronic amebic abscesses in man are often walled off by a dense fibrous capsule (Councilman and Lafleur 1891, Rogers 1922, Palmer 1938). In areas of the liver not directly involved with the necrosis, severe pericholangitis with dense infiltration of plasma cells was seen throughout the parenchyma. In amebic liver abscess, portal tracts may be infiltrated with lymphocytes and mononuclear cells (Palmer 1938). Extramedullary hematopoiesis was prominent in the experimental disease but has not been described in ALA.

Although liquefaction of the center often occurs in amebic liver abscess (Councilman and Lafleur 1891), amebic lesions in hamster liver usually remained solid two weeks after infection. In this study a large lesion caused by 200:NIH had fluid contents when a scalpel was used to cut into it. The solid nature and appearance of small amebic lesions in human liver caused Councilman and Lafleur (1891) to compare them with the tubercle. The apparent differences between the histology of amebic liver lesions in hamsters and man may be due to the length of time that lesions have been allowed to develop in their respective hosts. Lushbaugh et al. (1980b) put forth this theory when they observed formation of a fibrous wall and liquefaction of the necrotic center in hamster lesions that were allowed to progress four to six weeks. The same authors also suggested that lack of opportunity to study very early amebic lesions in human liver may account for a granulomatous reaction not having been described for amebic liver abscess (Aguirre-García et al. 1972, Bos and Hage 1975). The granulomatous inflammation seen in hamster liver may evolve into a fibrotic response more typical for amebic liver abscess in man (Lushbaugh et al. 1980b).

The host-parasite relationship in hepatic amebiasis of the hamster was a delicate balance that depended primarily

upon the pathogenicity of invading amebae as the deciding factor for the outcome of an experimental infection. An aggressive parasite like strain HM-1 was able to outstrip host defenses with an overwhelming proliferation of amebae, which were feeding on host cells and causing massive necrosis. Strain 200:NIH initially caused similar pathological changes. Being less pathogenic, 200:NIH amebae were unable to sustain a progressive necrotic process; and the host was able to repair most of the lesions by two weeks after infection, leaving mostly normal liver parenchyma. Repair of ALA involves regeneration of liver cells (Councilman and Lafleur 1891) and formation of connective tissue so that all traces of an amebic abscess may disappear from human liver (Kamat et al. 1970, Brandt and Pérez Tamayo 1970). Strains HU-21 and HK-9 were nonpathogenic and caused very little damage to hamster liver.

Persistence of Entamoeba histolytica was clearly necessary for sustained chronic inflammation in the liver of hamsters. The nature of this inflammation was a granulomatous reaction characteristically involving macrophages. Other inflammatory cells associated with amebic lesions in the hamster were polymorphonuclear leukocytes, eosinophils,

lymphocytes, and fibroblasts. Cellular reactions of this type suggest an immune response mediated by T lymphocytes (Unanue 1978).

Ultrastructural Changes in the Liver

The normal cytology of hamster liver is depicted in figure 40, an electron micrograph showing hepatocytes and a sinusoid. Figure 41 is a higher magnification of cytoplasmic organelles in an hepatocyte.

Electron micrographs were taken of HM-1 trophozoites in the liver of hamsters six days after portal vein infection. Unfortunately, most of the amebae were seen in areas of advanced necrosis, making difficult any conclusions about changes at the host-parasite interface. Figure 42 shows a trophozoite in the midst of necrotic debris.

Figure 43 shows ultrastructural changes in a less advanced lesion sampled six days after infection. The hepatocyte nucleus was intact, but there were fewer arrays of rough endoplasmic reticulum. Swollen mitochondria lost both their granules and cristae. Glycogen was depleted from the cell.

In figure 44 an ameba is wedged between two hepatocytes that show evidence of pathology. Although the nucleus in one of the liver cells is not visible in the area included within figure 44, it retained its double membrane. However,

the chromatin was denser than normal. Glycogen was severely depleted and swollen mitochondria lost their typical architecture. Endoplasmic reticulum was dilated, and the ground substance in one part of an hepatocyte was nearly devoid of organelles. The plasma membrane of liver cells was no longer intact. At one end of the ameba, its plasmalemma appeared discontinuous, possibly permitting cell contents to spill out.

Cytoplasmic organelles in hepatocytes appeared more susceptible to damage by amebae than the nucleus, at least initially. Endoplasmic reticulum, mitochondria, and glycogen stores all showed early signs of pathology. Progressive ultrastructural changes led to destruction of the plasmalemma and death of the host cell. Comparable description of ultrastructural pathology in human ALA is not available.

Ultrastructure of Entamoeba histolytica in Situ

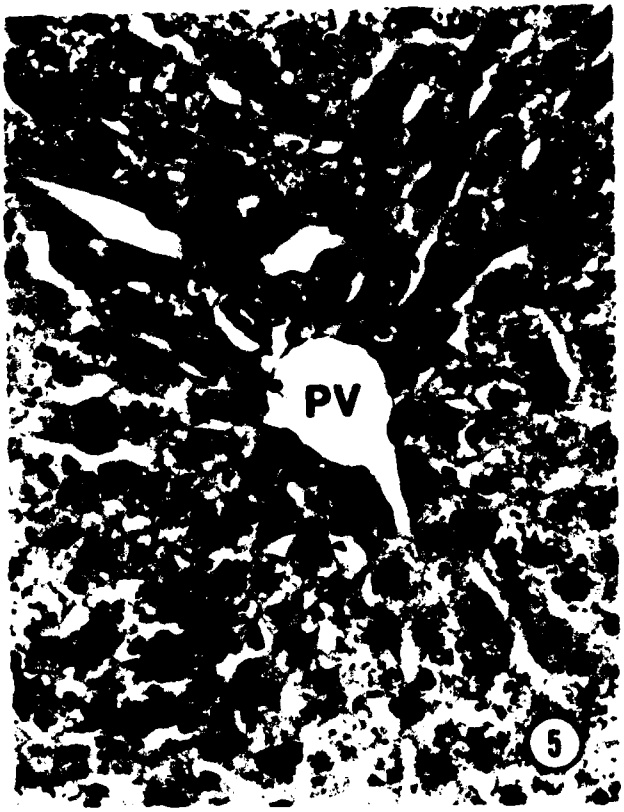
The trophozoite in figure 45 illustrates the feeding habits of Entamoeba histolytica. This ameba had already ingested some erythrocytes and was ingesting a granulocyte at the time of fixation. Notice the phagocollar through which the leukocyte was being taken into the parasite's cytoplasm. Remains of leukocytes were not uncommon in food vacuoles of trophozoites (figure 46).

One of the most intriguing features of E. histolytica is the surface active lysosome (figure 47), now regarded by Lushbaugh and Pittman (1979) as a filopodium that is sometimes associated with a vacuole just below the plasma membrane. Filopodia (figure 48) have a trilaminar membrane that is continuous with the plasmalemma. A fuzzy coat or glycocalyx covers the surface of the plasmalemma and filopodia.

The cytoplasmic features of trophozoites in situ agreed with published accounts (appendix 1) whether the amebae had come from man, experimental animals, or cultures. The whorls inside food vacuoles were distinctive (figure 44). Short ribosomal helices (figure 46) and endoplasmic reticulum were evident. Glycogen was abundant in all the trophozoites and often appeared in rosettes. The inset in figure 47 suggested a connection between tubular channels and cytoplasmic vacuoles. Virus particles were not seen in any electron micrographs of amebae in this study.

Not all amebae survived in the hamster liver. Figure 49 shows a dead trophozoite with enough ultrastructural detail to identify it among necrotic host material.

Figure 5. Normal liver in LHC/Lak hamster. Trabeculae radiated from a branch of the portal vein (PV) and surrounded the sinusoids (S). Cuboidal epithelium lined the bile duct (BD), which was accompanied by a branch of the hepatic artery and a lymphatic channel in the portal tract. The latter two vessels were not visible in this plane of section. H&E, X400.


PV
5

Figures 6-30 depict the microscopic and gross pathology of experimental hepatic amebiasis caused by axenically cultivated Entamoeba histolytica strain HM-1. From 50 to 315 thousand trophozoites were inoculated into the portal vein, and necropsy followed 1-15 days later.

Figure 6. Day-old amebic lesion near the surface of the liver. Resembling an infarct with an inflammatory center, this lesion probably resulted from am embolus of amebae that became trapped in a small branch of the portal vein. H&E, X100.

Figure 7. Enlargement of figure 6. Granulocytes (G) and mononuclear cells (M) have begun to infiltrate the necrosis caused by amebae (A). H&E, X400.

6
7

Figure 8. Hematophagous trophozoite (A) with a typical Entamoeba nucleus. This ameba was seen with numerous others in a necrotic area of a 5-day lesion. H&E, X1000.

Figure 9. Large multilocular lesion resulting from coalescence of smaller ones. Hepatocytes at the upper left were compressed, and the portal tract (P) showed mild inflammation. Dystrophic calcification (C) was already evident in the necrotic part of the lesion. 3 days, H&E, X100.


8
9

Figure 10. Sparse layer of fibroblasts (F) between two adjacent lesions. Notice the ameba (A) near a layer of mononuclear cells. 3 days, H&E, X250.

Figure 11. Numerous trophozoites (A) concentrated in a feeding zone between necrotic tissue on the left and normal liver parenchyma on the right. 5 days, PAS, X100.

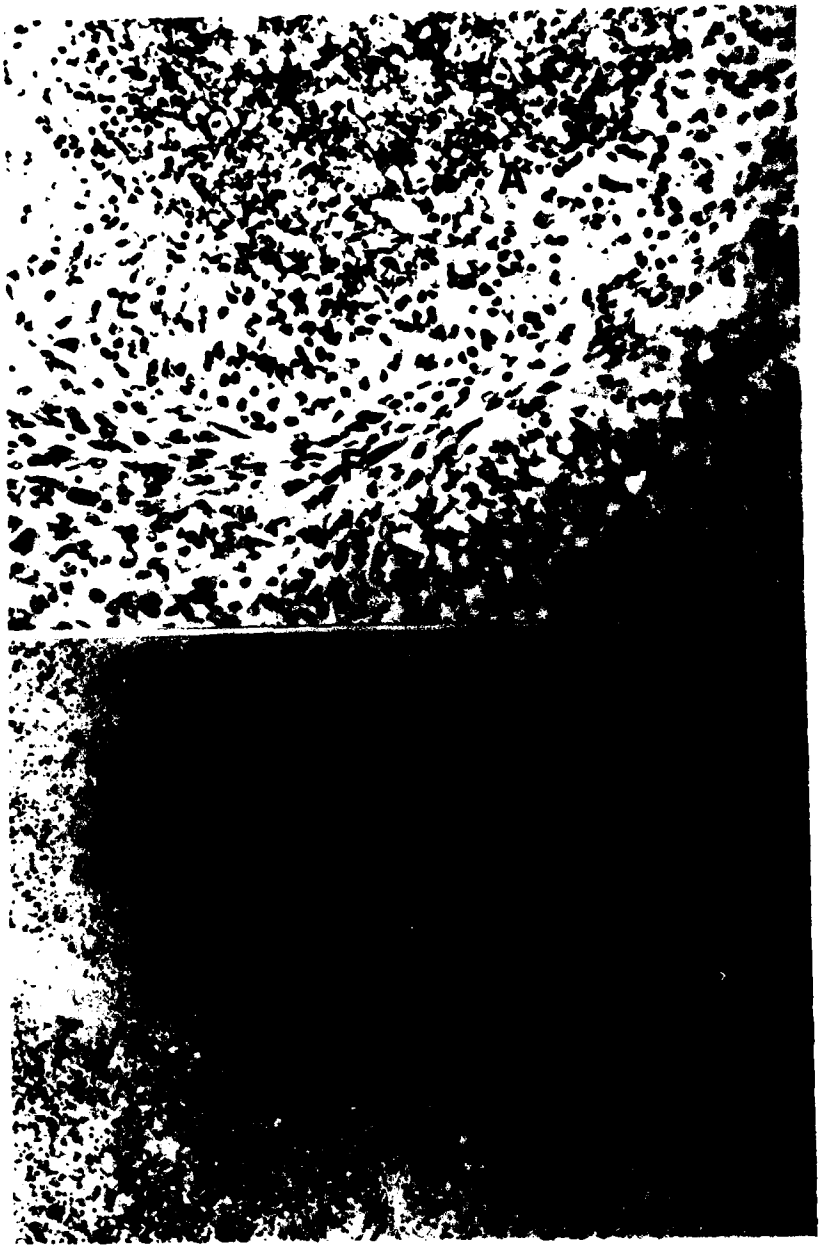

A

Figure 12. Secondary focus of amebic invasion near a more advanced, multilocular lesion on the left. Amebae spread beyond initial sites of infection by direct extension to adjacent areas or by the hematogenous route (cf figure 16). 5 days, H&E, X100.

Figure 13. Gross pathological changes in the liver seven days after intraportal inoculation with 50 thousand HM-1 amebae. The large nodular lesions were yellowish and had a firm consistency. Scale interval 1 mm.

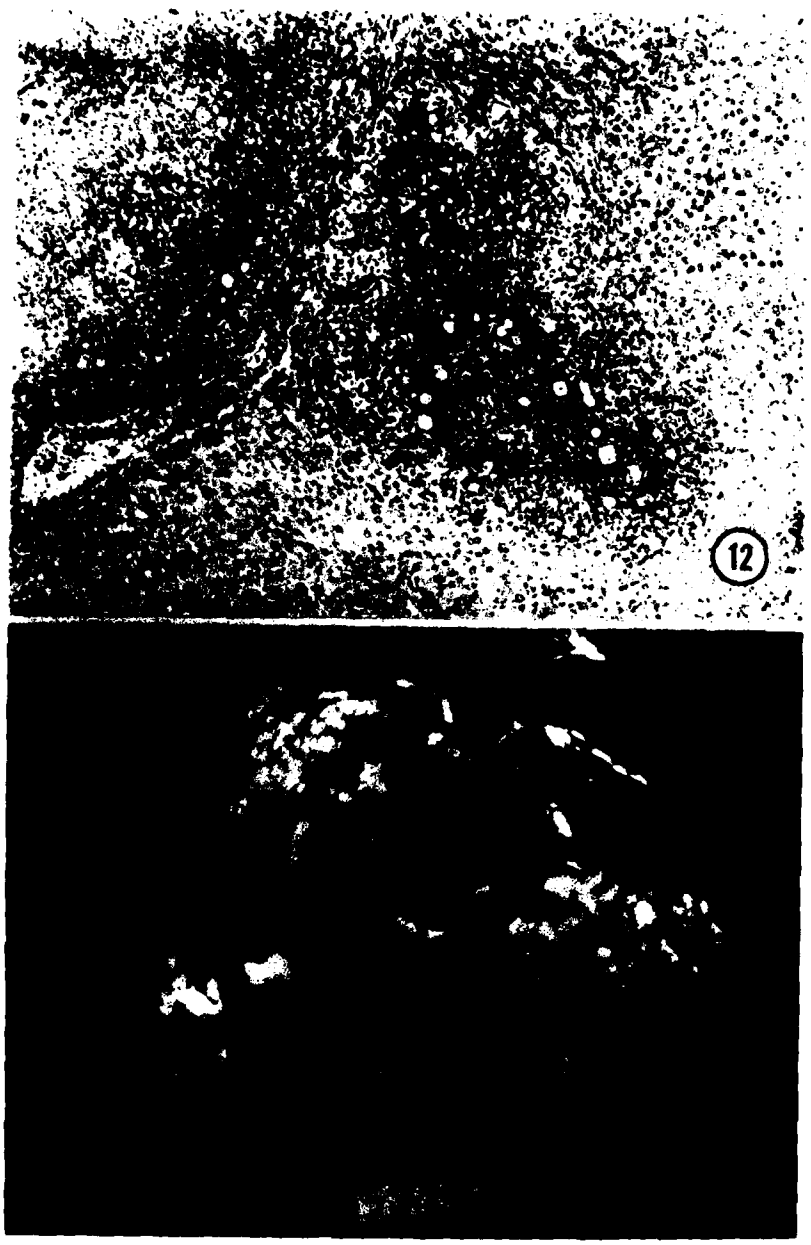
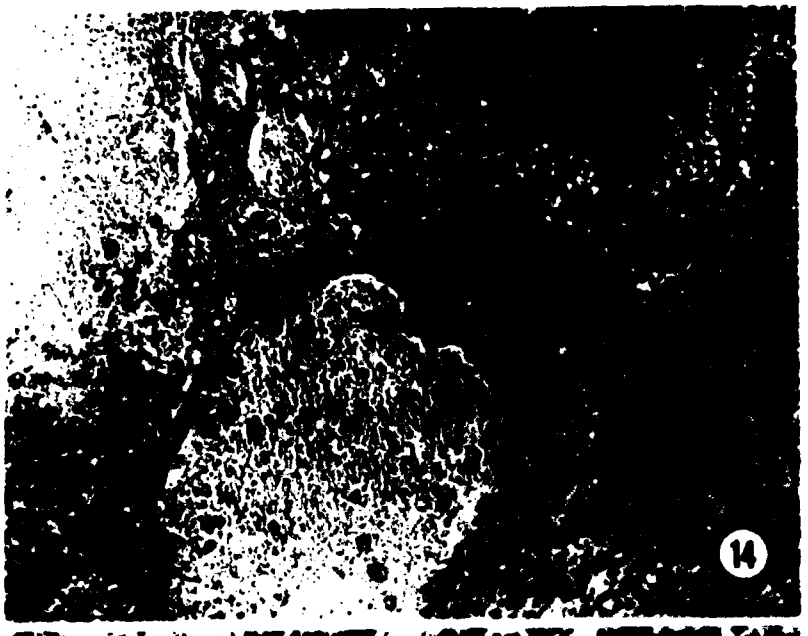

12

Figure 14. Extensive necrosis of the liver caused by pathogenic amebae. Granulocytes and mononuclear cells infiltrated the edges of lesions where macrophages were accumulated. 7 days, H&E, X100.

Figure 15. Granulomatous chronic inflammation of an amebic lesion at seven days. Macrophages (M) were the principal inflammatory cells as fibroblasts (F) attempted to wall off the injury; but other mononuclear cells, presumably lymphocytes, also infiltrated the lesions. H&E, X400.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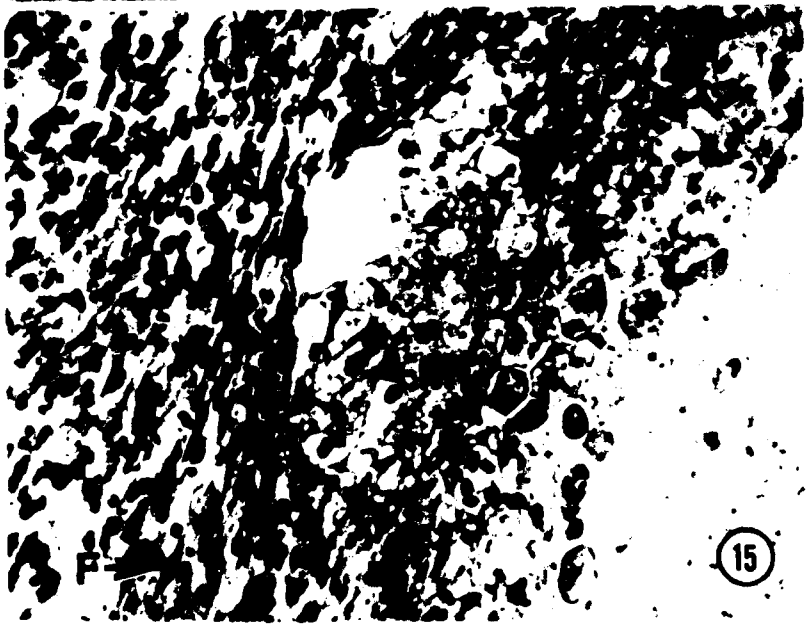
15

Figure 16. Trophozoite (arrowhead) inside a blood vessel partially surrounded by a necrotic lesion. 7 days, H&E, X100.

Figure 17. Proliferation of bile ducts (BD) in response to injury. A layer of macrophages (M) surrounding the necrotic center of an amebic lesion was particularly prominent in this lesion. 7 days, H&E, X100.


16

Figure 18. Extramedullary hematopoiesis and periportal inflammation of the liver. Numerous bands or immature granulocytes were in the parenchyma while mononuclear cells and eosinophils were concentrated near the portal vein. Notice margination of leukocytes in the vein. 7 days, H&E, X100.

Figure 19. Extramedullary hematopoiesis in the spleen of a hamster with extensive amebic necrosis of the liver nine days after infection. H&E, X400.

Figure 20. Thrombophlebitis of the portal vein. Amebae may have broken out of the portal vein at the site indicated by arrowheads to produce the necrotic lesion visible in the upper left corner. 9 days, H&E, X60.

Figure 21. Gross pathological changes in the liver 11 days after infection with 315 thousand pathogenic amebae. Much of the liver was necrotic and some nodular lesions extended through the thickness of the liver. Scale interval 1 mm.

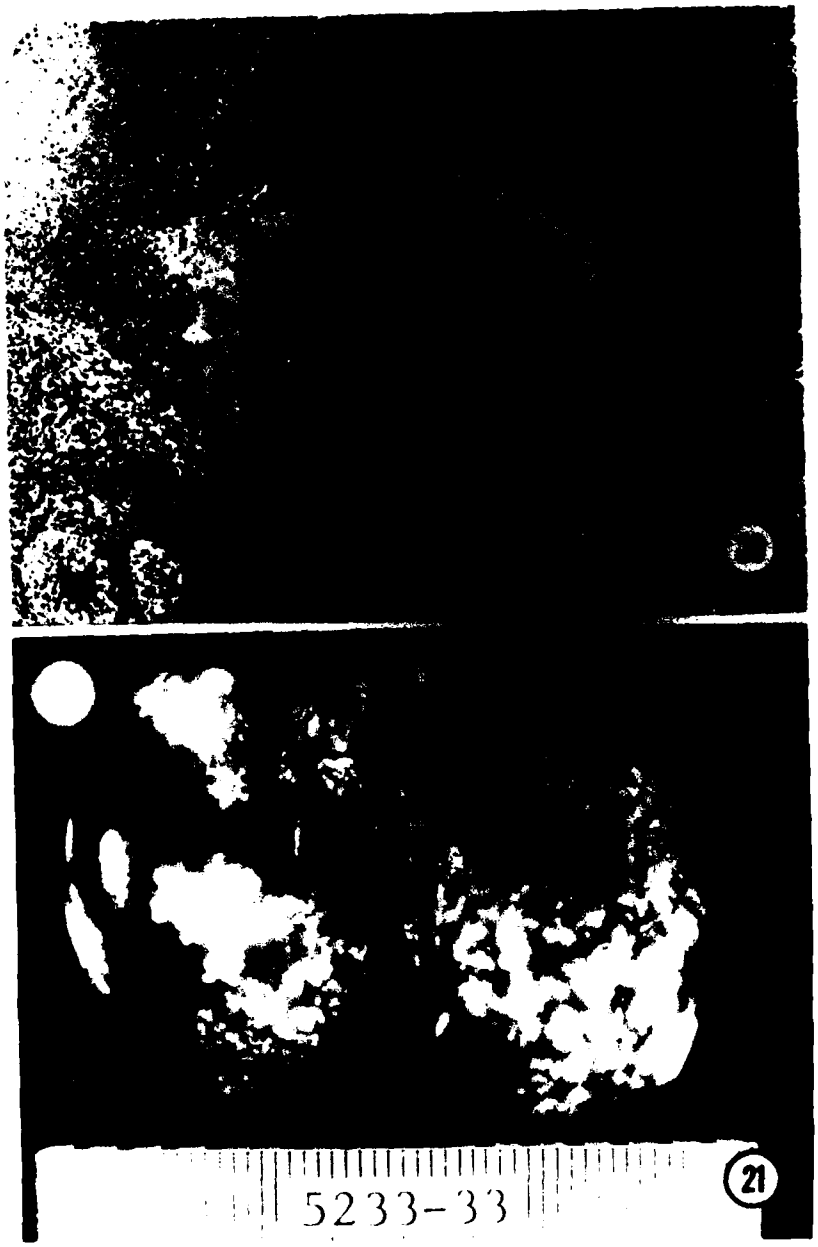
5233-33
21

Figure 22. Low power magnification of amebic lesions in hamster liver 11 days after infection. In this area, normal tissue was absent between lesions. Chronic inflammation and fibrosis surrounded areas of caseous necrosis. H&E, X40.

Figure 23. Granulomatous reaction to Entamoeba histolytica. Fibrosis (F) became a prominent part of the host response by 11 days when numbers of trophozoites (A) had increased immensely. H&E, X100.

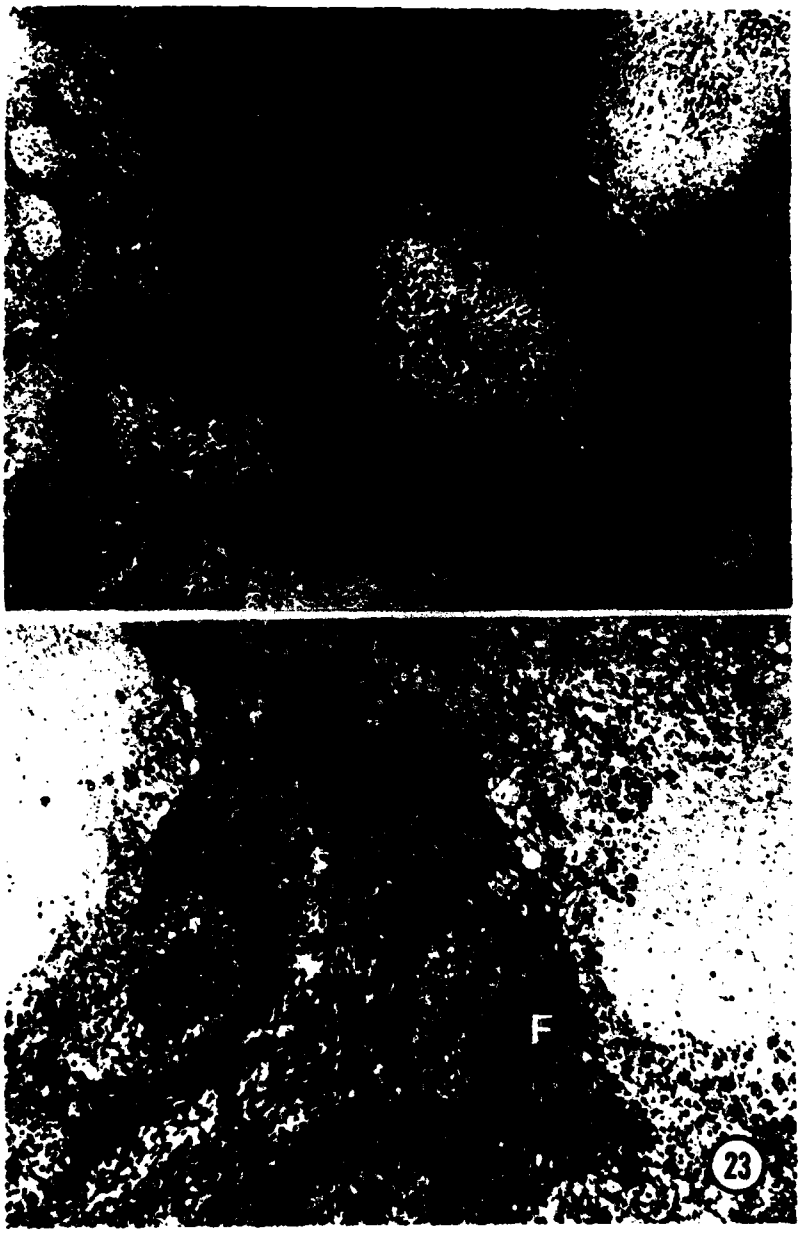
F
23

Figure 24. Plasma cells (P) and lymphocytes in a portal tract 11 days after amebic infection of the liver. Note the mitotic figure (arrow) and large number of immature lymphocytes. H&E, X400.

Figure 25. Pericholangitis associated with hepatic amebiasis in the hamster. Lymphocytes and plasma cells (P) were the major inflammatory cells surrounding the bile duct (BD), arteriole (AR), and lymphatic (LY). This histological picture suggested local antibody production. 11 days, H&E, X400.

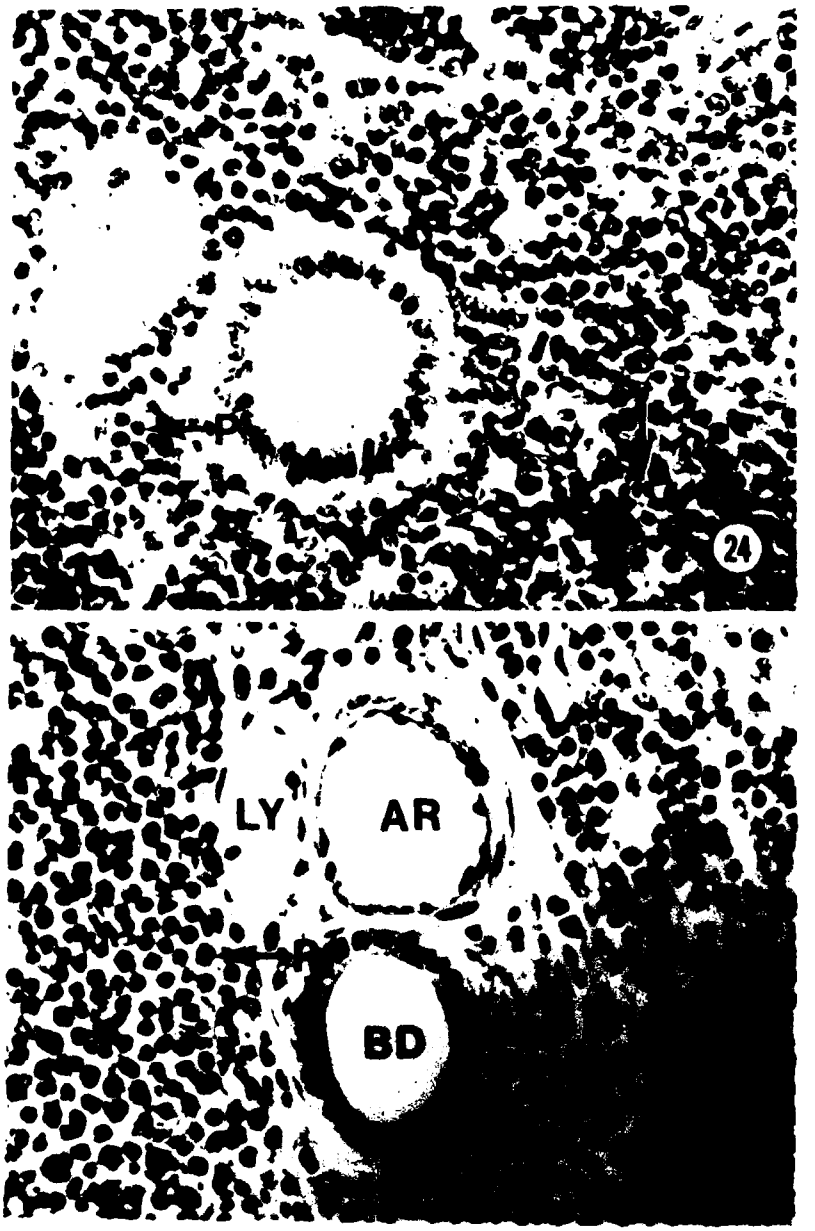
24
LY
AR
BD

Figure 26. Caseous necrosis of the liver caused by Entamoeba histolytica 15 days after infection via the portal vein. This liver was approximately twice normal size, and some lobes were occupied almost entirely by amebic lesions. Scale interval 1 mm.

Figure 27. Numerous amebae in a necrotic lesion 15 days after infection. Mononuclear cells were prominent in the granulomatous cellular reaction. H&E, X100.

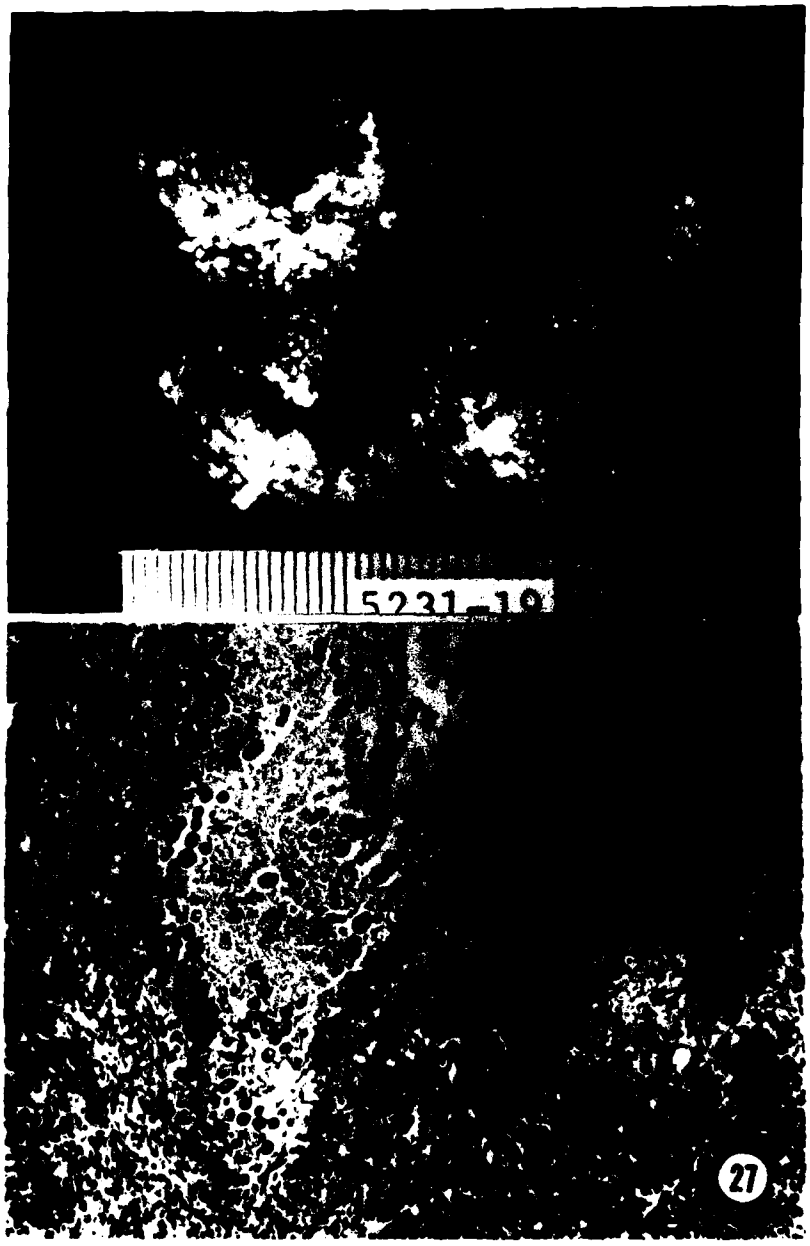
27

Figure 28. Masses of amebae were characteristically found in 15-day HM-1 lesions. PAS, X100.

Figure 29. Fibroplasia as part of chronic inflammation to amebic invasion of the liver. 15 days, H&E, X250.

A

Figure 30. Numerous trophozoites (A) in a large blood vessel. Hematogenous spread of amebic infection throughout the liver was very common late in the disease when host defenses were overwhelmed. 15 days, H&E, X100.

Figures 31-36 show pathological changes in the liver of hamsters that received 50-315 thousand axenic strain 200:NIH trophozoites via the portal vein.

Figure 31. Histopathological changes caused by 200:NIH but identical to those caused by strain HM-1 at 11 days. Compare figure 22. H&E, X40.

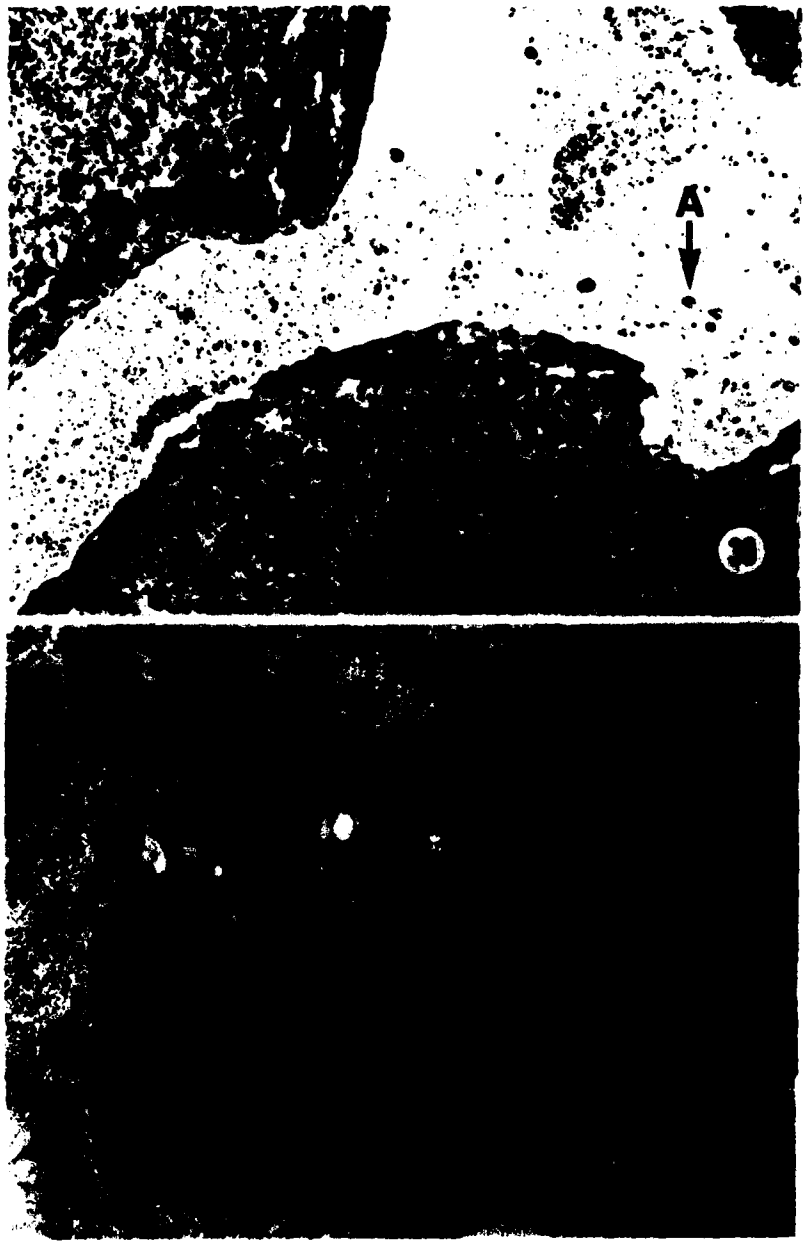
A

Figure 32. Chronic inflammation and extramedullary hematopoiesis in response to hepatic infection with 200:NIH. 11 days, H&E, X100.

Figure 33. Granulomatous reaction. Note the layer of macrophages (M) surrounded by fibroblasts (F) and numerous mononuclear cells. 11 days, H&E, X250.

32
33

Figure 34. Microabscess consisting of polymorphonuclear leukocytes and amebae (not shown). 11 days, H&E, X250.

Figure 35. Repair of an amebic liver lesion caused by 200:NIH. Granulation tissue in the middle of a necrotic lesion contained many fibroblasts, mononuclear inflammatory cells, and an occasional giant cell (arrow). Amebae were not seen where healing had begun. 7 days, H&E, X100.

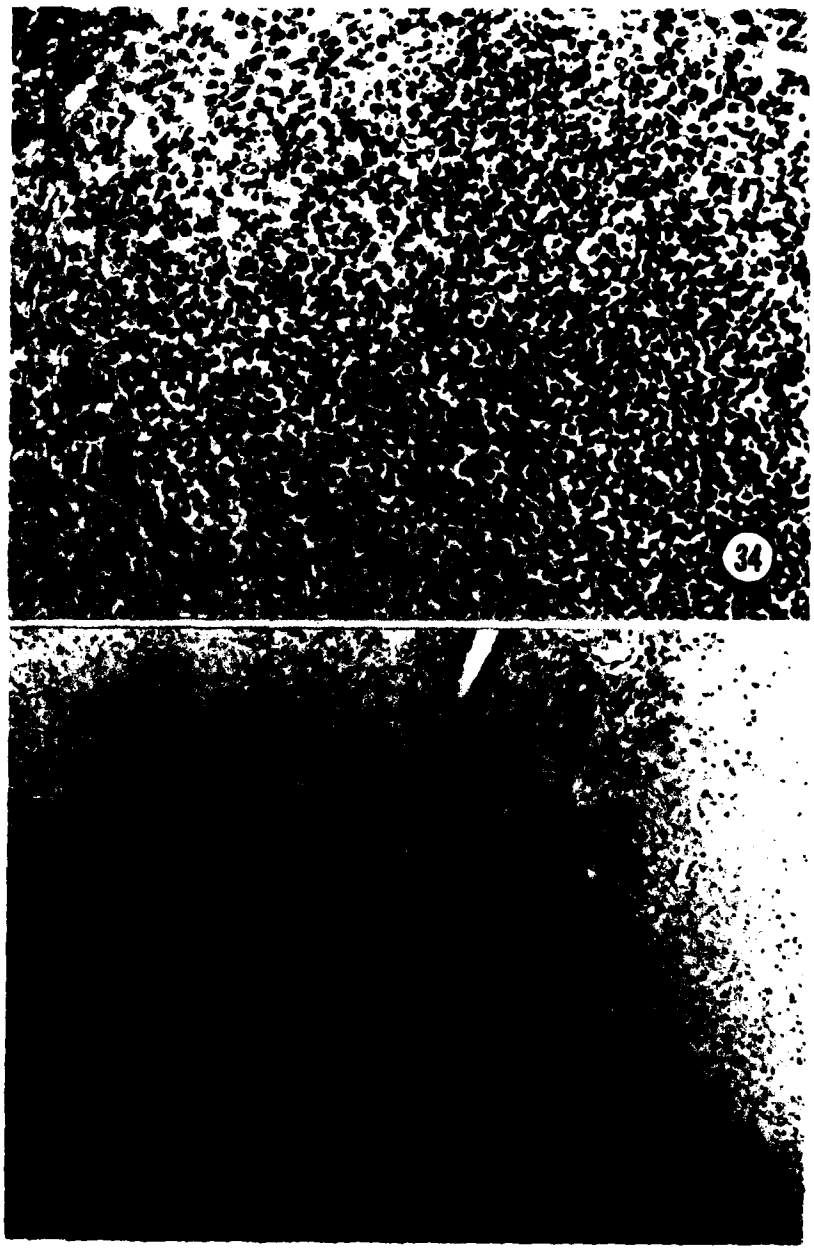
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Figure 36. Healing lesion 15 days after infection with 200:NIH. H&E, X100.

Figure 37. Hepatocellular degeneration with infiltration by mononuclear cells in a hamster that received 50 thousand trophozoites of strain HK-9. No amebae were seen in PAS sections. 3 days, H&E, X250.


36

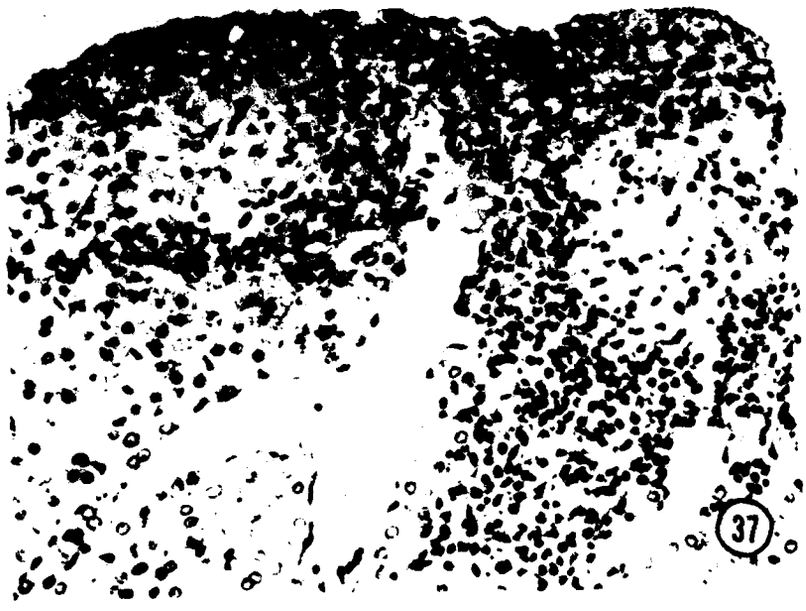
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Figure 38. Active amebic lesion caused by strain HU-21 at seven days. Several trophozoites (arrowhead) were found in necrotic areas, which were surrounded by a granulomatous reaction as in HM-1 lesions (cf figure 16). H&E, X100.

Figure 39. Healing lesion in the liver of the same animal that had the lesion depicted in figure 38. 7 days, H&E, X100.

38

Figure 40. Normal hepatocytes from an LHC/Lak hamster. Bile canaliculus (BC), endoplasmic reticulum (ER), glycogen (GL), mitochondrion (M), microvilli (MV), nucleus (N), sinusoid (S).

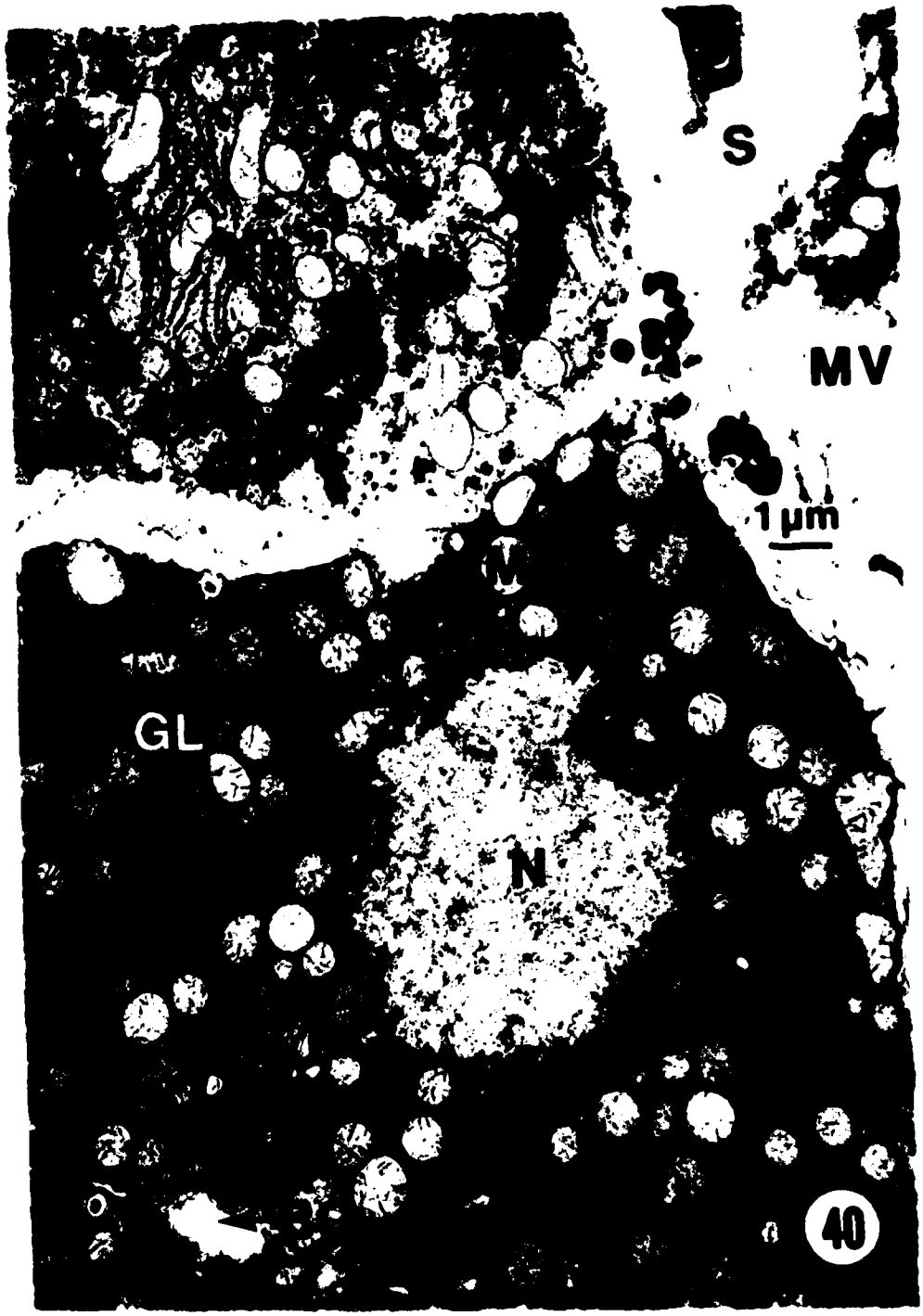
S
MV
1 µm
M
GL
N
40

Figure 41. Cytoplasmic organelles of a normal hepatocyte from the hamster. Bile canaliculus (BC), crista (CR), desmosome (D), endoplasmic reticulum (ER), Golgi complex (G), glycogen (GL), mitochondrion (M).

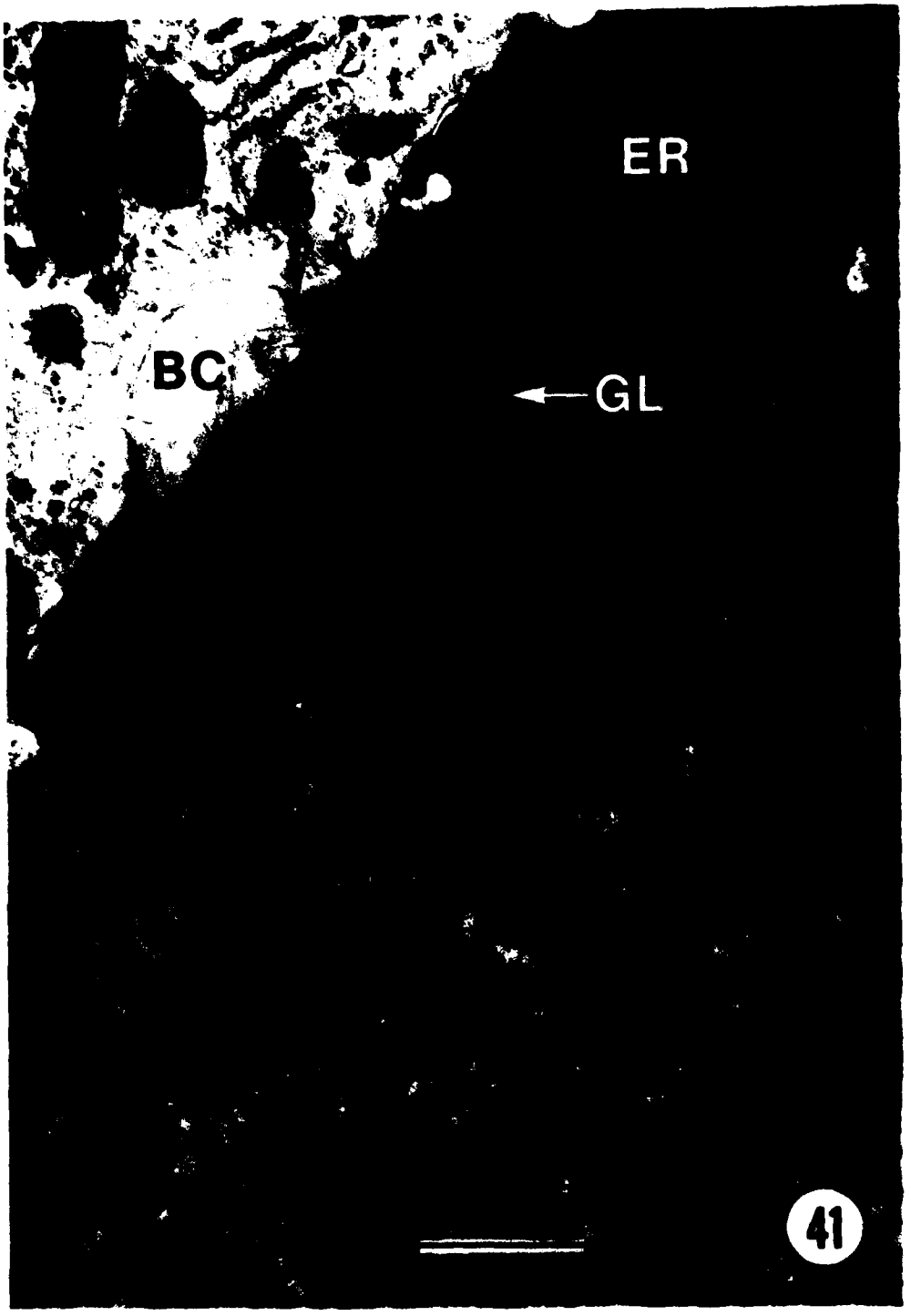
ER
BC
GL
41

Figure 42. Axenic trophozoite of Entamoeba histolytica strain HM-1 in hamster liver six days after infection. The nucleus (N) of this ameba had an unusual shape, and some of the ruffled surface features were proximal ends of filopodia (arrow). Food vacuole (V).


1 µm
N
42

Figure 43. Early ultrastructural changes in an hepatocyte involved in hepatic amebiasis. Although the nucleus (N) appeared intact, mitochondria (M) were swollen and had lost their cristae. Arrays of rough endoplasmic reticulum were less numerous.

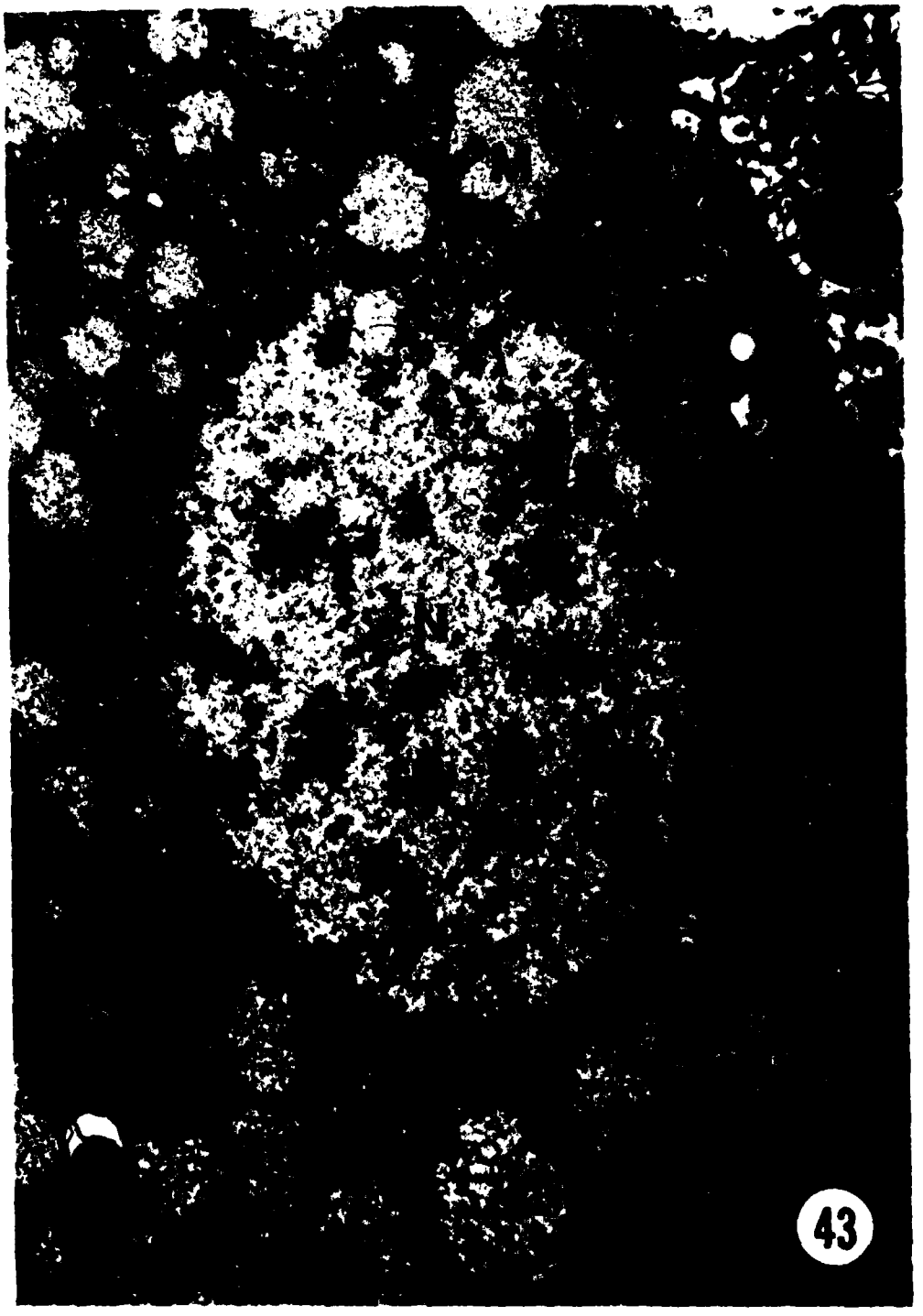
43

Figure 44. Trophozoite (A) between two hepatocytes. In addition to swollen mitochondria, the endoplasmic reticulum (ER) of one hepatocyte was dilated in an area where the ground substance had lost most of the organelles. The plasmalemma of the ameba was discontinuous at one end (arrowheads) where the cell contents spilled out.


4

Figure 45. Entamoeba histolytica trophozoite (A) ingesting a granulocyte (G). The ameba had already ingested some erythrocytes (RBC). Notice the phagocollar (P) through which the host cell was taken into the parasite. Nucleus (N).

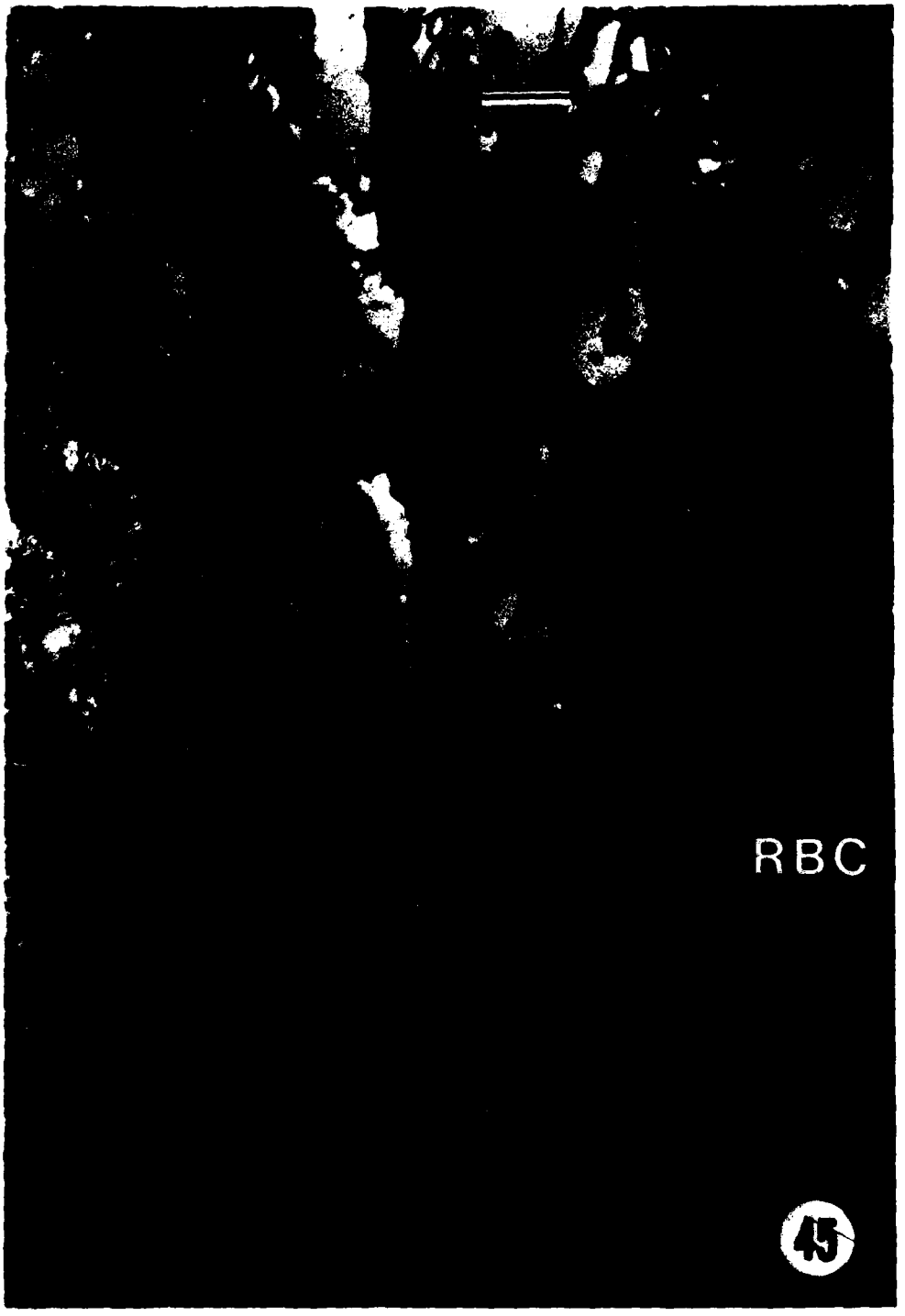
RBC
43

Figure 46. Cytoplasmic features of a trophozoite in situ. Glycogen (GL), cytoplasmic granules of an ingested granulocyte (GR), ribosomal helix (R).

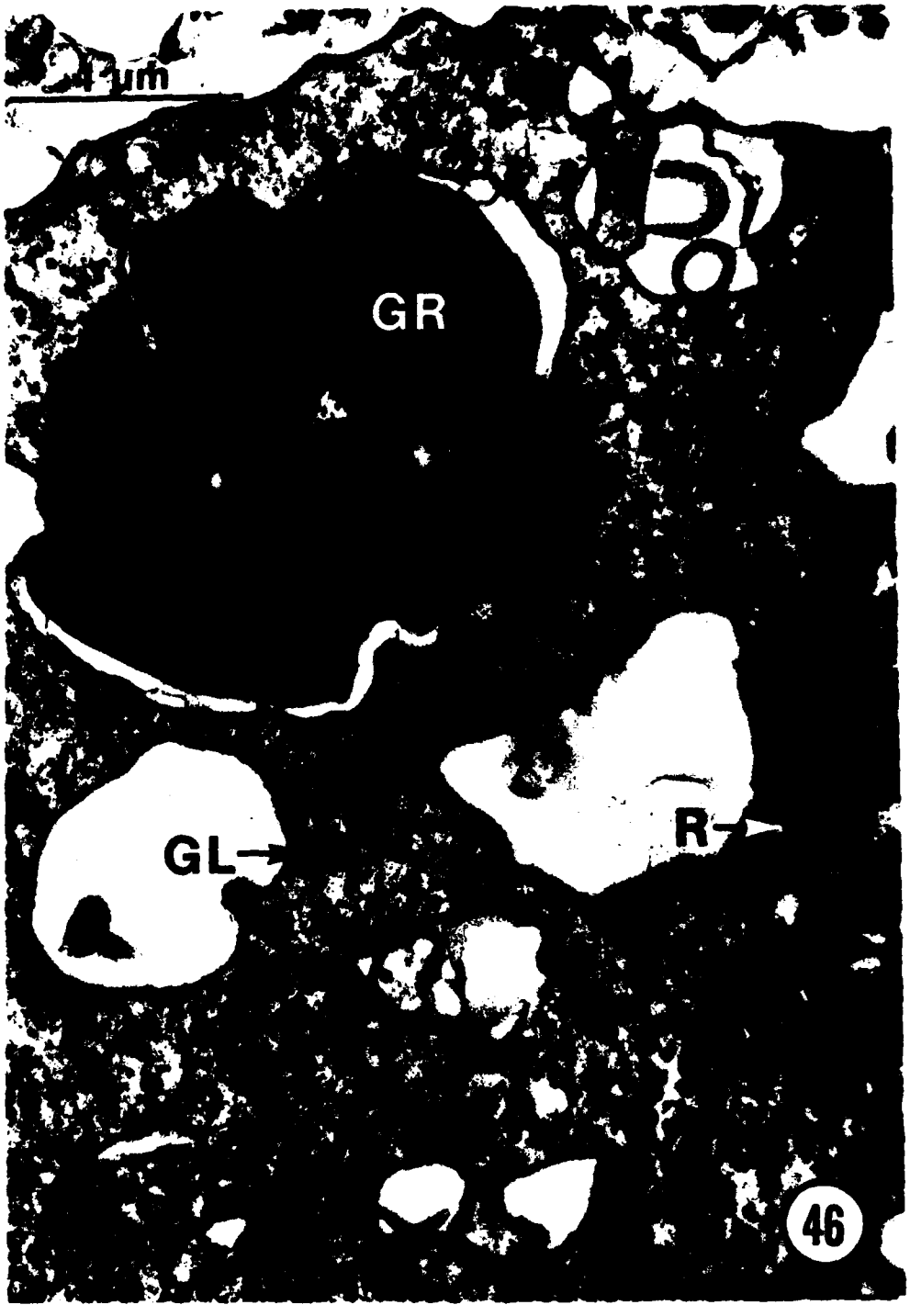
1 µm
GR
GL
R
46

Figure 47. Fine structure of trophozoite cytoplasm. The inset shows detail of a region suggesting a connection between a vacuole (V) and tubular channels (arrow). So-called surface active lysosome (SAL).

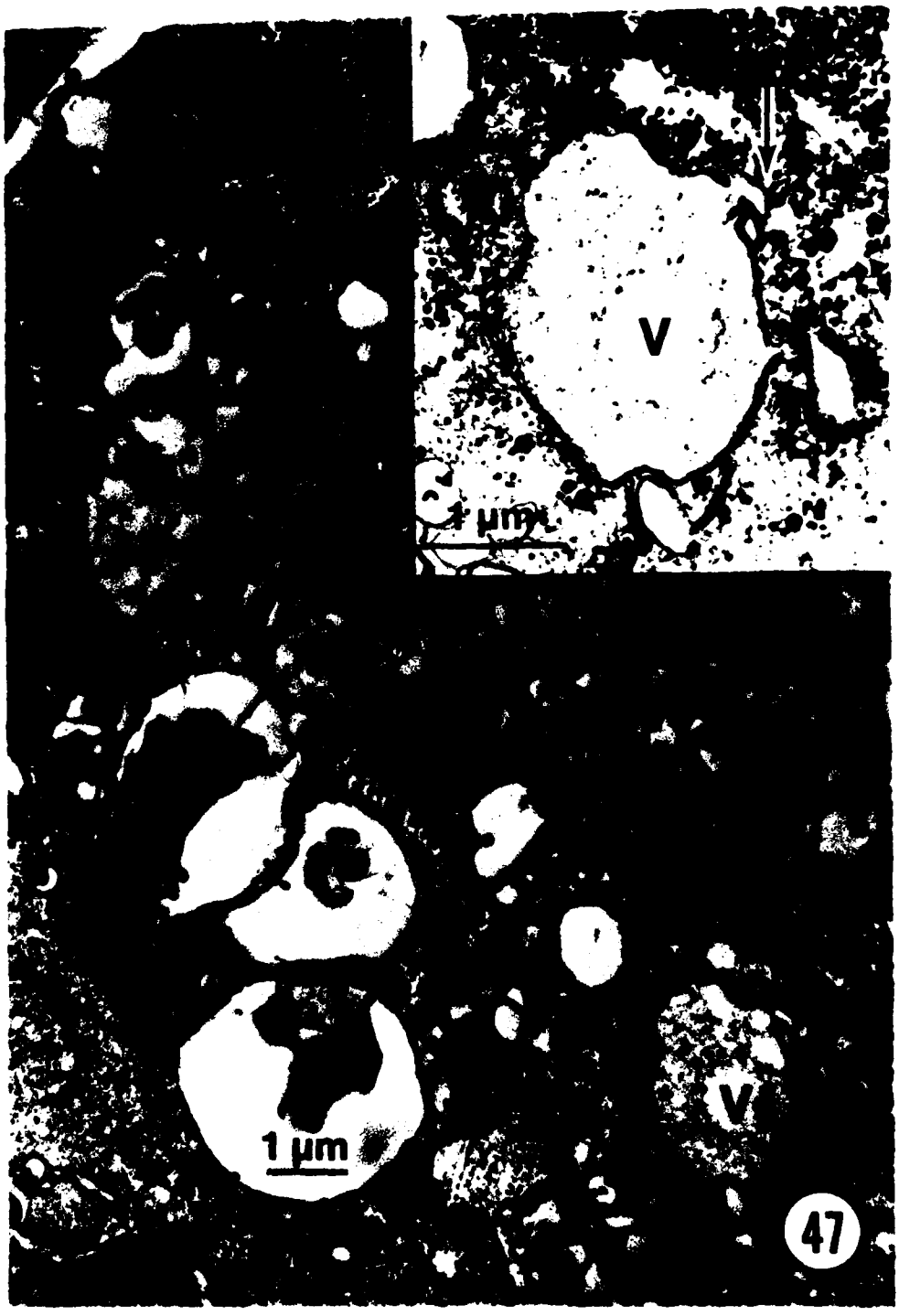
V
1 µm
1 µm
V
47

Figure 48. Surface features of Entamoeba histolytica trophozoite. Filopodia (arrowheads) were commonly seen at the surface of amebae in situ. Part of a host leukocyte (WBC) was visible in this field.

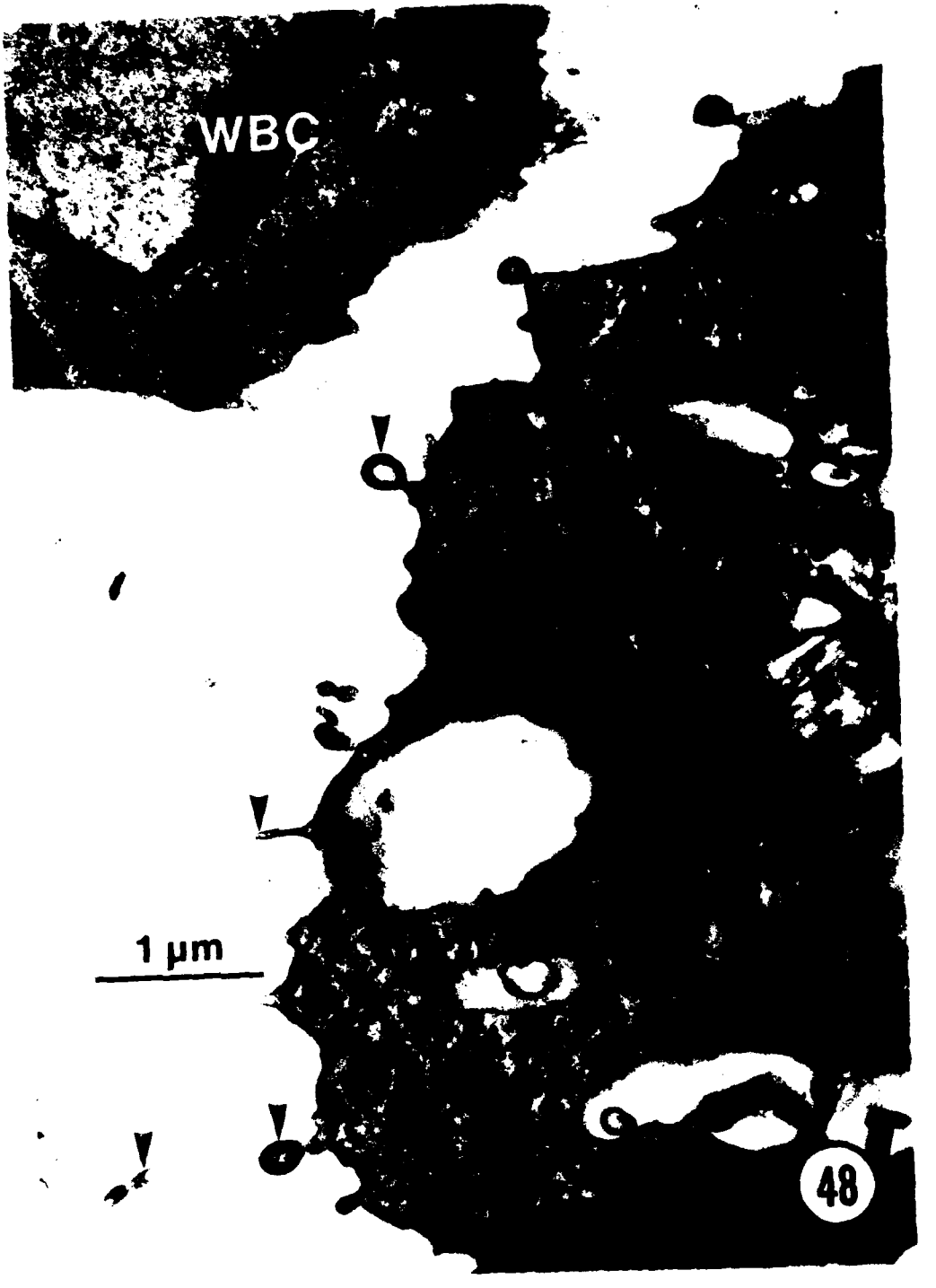
WBC
1 µm
48

Figure 49. Dead trophozoite (A) in necrotic host tissue.


49

Pathophysiology

In addition to pathology, a variety of pathophysiological changes was studied in the hamster model.

Body Weight

Change in body weight after inoculation was a means of assessing the overall health and condition of experimental animals. In the analysis of variance (table 14) time was the only significant main effect. As one can see from figure 50, all of the animals experienced a dramatic loss of body weight within three days following surgery. They recovered to their original body weight within 15 days, and this trend over time was linear (table 14). Because all of the animals behaved in a similar manner and controls did not differ significantly from infected animals with respect to change in body weight, this variable seemed unrelated to amebic infection. The initial loss of body weight was obviously due to surgical intervention and the trauma associated with it. However, animals with the most severe disease (HM-1 group) had livers that doubled in weight (figure 3). In those animals the added weight due to hepatomegaly brought their total body weight up to normal. Therefore, severe hepatic amebiasis in hamsters did cause loss of body weight after accounting for enlargement of the liver.

Hematology

The blood picture is a good way to study the host response to an infectious disease. Erythrocyte counts, microhematocrit, leukocyte counts, and differential counts were done on blood samples taken just prior to necropsy.

A significant strain x dose interaction was detected by analysis of variance in RBC count (table 15). Figure 51 indicates no interaction for strains 200:NIH, HU-21, and HK-9. The interaction was due to HM-1, especially at higher dose levels. In other words, the strain x dose interaction would be absent except for one level (HM-1) of the strain factor. Cox (1958) discussed the interpretation of similar interaction terms in ANOVA.

Animals infected with higher doses of HM-1 were anemic compared to animals infected with other strains and sham-operated controls (figure 51, table 15). These data also showed a linear trend over time for RBC count (table 15). Duncan's multiple range test confirmed that HM-1 was significantly different from the other strains at the .01 level when controls were excluded from the strain means.

A similar strain x dose interaction was at play in the data for microhematocrit (figure 52, table 16). As with the RBC count, HM-1 was significantly different from the other strains. One can see from figure 52 that 200:NIH infected

animals had hematocrits intermediate between HM-1 and the other strains, especially at the higher dose levels. The Duncan multiple range test reflected this situation by finding HM-1 significantly different from HU-21 and HK-9 at the .01 level but not different from 200:NIH. Neither was 200:NIH significantly different from HU-21 or HK-9 by the Duncan procedure. The main effect of time was responsible for significant variation, and the combined hematocrit data for all strains and doses displayed a linear trend with time (table 16). The dose effect showed a statistically significant difference between control animals and infected ones.

The HM-1 group became anemic and so did animals infected with 200:NIH at the highest dose level. Animals infected with the other two strains of amebae did not become anemic compared to controls.

The behavior of ameba strains was somewhat different for leukocyte count. The analysis of variance in table 17 indicated that HM-1 was not significantly different from the other strains. Strain 200:NIH caused a greater leukocytosis than HM-1 and had its peak between seven and 11 days after infection (figure 53). Both HM-1 and 200:NIH caused lower WBC counts 15 days after infection than earlier in the

experiment (figure 53). Time was a significant source of variation in the experiment, but a straightforward interpretation of a temporal trend was impossible with ANOVA (table 17). Control animals had significantly lower white counts than infected ones (table 17), and strains 200:NIH and HM-1 caused more severe leukocytosis with increasing dose of amebae (figure 54). A posteriori comparison of the strain means indicated that 200:NIH and HM-1 did not differ significantly in WBC count. The difference between strain 200:NIH and strain HK-9 or HU-21 was significant only at the .05 level by the Duncan procedure. These data indicated that leukocytosis was caused by 200:NIH and HM-1 amebae. The leukocytosis increased with the dose of these two strains and became more severe between seven and 11 days after infection. By 15 days after infection the white count approached the level in control animals.

Analysis of the differential blood counts revealed surprisingly few significant differences. For example, analysis of variance in the percentage of neutrophils and lymphocytes revealed no significant main effects or interactions. By comparing figure 55 with figure 54, one can readily see that the actual number of neutrophils was largely responsible for the WBC count. Animals infected with HM-1 or 200:NIH had more neutrophils (figure 55) but

proportionately fewer lymphocytes (figure 56) than animals infected with the other two strains, although the difference was not statistically significant. Band neutrophils were not tallied separately.

The percentage of monocytes showed a linear decrease with time (table 18, figure 57). The only significant difference among the strains was between 200:NIH and HU-21, the strains with the highest and lowest numbers of monocytes, respectively. This difference was supported by the Duncan test being significant for that comparison at the .01 level. Control animals did not differ significantly from infected ones with respect to monocytes.

Although the percentage of eosinophils on blood smears did not have any sources of variation significant at the .01 level, the actual number of eosinophils in the peripheral blood had significant variation due to strain and time effects (table 19). Strain 200:NIH was related to higher eosinophilia than the other strains, but this difference was significant only when 200:NIH was compared with HU-21 (table 19). The difference between eosinophilia with 200:NIH and strains HU-21 or HK-9 was significant only at the .05 level by Duncan's procedure. There was a significant time effect (table 19) due to an obvious quadratic trend in figure 58.

Both 200:NIH and HM-1 produced eosinophilia on day seven after infection, but the number of peripheral eosinophils returned near normal by day 15.

The existence of basophils in the peripheral blood of hamsters has been debated for a number of years (Hoffman et al. 1968). In this study, strain HM-1 was the only one capable of eliciting basophils in the peripheral blood of infected hamsters (figure 59). Several of the animals infected with HM-1 had 1% basophils on their blood smears collected three to 11 days after infection. None of the other animals nor sham-operated controls had cells identifiable as basophils on blood smears.

The blood picture of hamsters with severe hepatic amebiasis was quite similar to that of patients with amebic liver abscess. Hamsters infected with HM-1 developed anemia, which was more severe at higher doses of amebae. High doses of 200:NIH were required to produce a noticeable degree of anemia. ALA patients often have an absolute neutrophilia, which diminishes as the lesion becomes chronic; but they do not have eosinophilia (Ochsner and DeBakey 1943). Likewise, animals given HM-1 or 200:NIH had a dose-related leukocytosis caused by an increase in the actual number of neutrophils. Both HM-1 and 200:NIH caused leukocytosis one week after infection, and the WBC count diminished to almost

normal at 15 days. Eosinophilia was not a significant part of the blood picture in experimental hepatic amebiasis. Basophils were seen only in the peripheral blood of hamsters with severe disease caused by HM-1.

Serum Proteins

Protein electrophoresis was done on serum samples from controls and infected hamsters. The amount of total protein in the serum did not differ among strains or doses of amebae or among times after infection.

The ratio of albumin to globulin in the serum had several sources of variation (table 20). Analysis of variance suggested a strain x time interaction, the usual one due to HM-1 (table 21). Hamsters infected with HM-1 had A:G near or below 1.0, whereas animals infected with the other strains had A:G greater than 1.0 with the exception of 200:NIH at 11 days. Therefore, 200:NIH also contributed to the strain x time interaction. The strain x dose interaction suggested by ANOVA (table 20) did not occur between strains HU-21 and HK-9 (figure 60). This interaction was due entirely to the behavior of strains 200:NIH and HM-1, which had lower A:G ratios than the other strains. The lower A:G ratios caused by HM-1 were significantly lower than those of the other strains (table 20). The Duncan procedure did not detect a significant difference between

HM-1 and 200:NIH, but HM-1 was significantly different from HU-21 and HK-9 at the .01 level. A definite trend with time was not obvious by analysis of variance (table 20), but the analysis did suggest a nonlinear function. Statistical significance of the dose factor in the experiment (table 20) was due to a highly significant difference between control animals and infected ones. Further examination of albumin and globulin fractions will explain these data on A:G ratio.

The analysis of variance in albumin suggested both a strain x time and strain x dose interaction (table 22). The data in table 23 indicate that no interaction occurred between strains HU-21 and HK-9. On the other hand, HM-1 and 200:NIH had less albumin than the other groups; HM-1 and 200:NIH had similar levels over time except late in the infection. Figure 61 explains the strain x dose interaction by the lower amounts of albumin in animals infected with 200:NIH or HM-1 compared to control animals or those infected by the other two strains. Analysis of variance confirmed that infected animals had significantly less albumin than uninfected controls (table 22). There was no significant trend with time. The highly significant strain effect was due to the much lower levels of albumin caused by HM-1 compared to the other strains. This finding held true by the Duncan procedure at the .01 level. The amount of

albumin in the serum decreased when greater numbers of HM-1 or 200:NIH amebae were given (figure 61).

In the globulin fraction, alpha-1 and alpha-2 globulins did not show any significant differences related to disease. Uninfected controls and experimentally infected hamsters had similar levels for those two globulin peaks.

In a normal or uninfected control animal, beta and gamma globulin peaks (figure 62A) were usually demarcated well enough to make the scanning of the electrophoretic pattern fairly simple. The electrophoretic pattern for serum from an animal with elevated gamma globulin (figure 62B) presented a more difficult problem in separating beta and gamma globulin because the two peaks tended to merge.

Analysis of variance in beta globulin (table 24) suggested a strain x dose interaction and showed that uninfected controls had less beta globulin than infected animals. Animals infected with HM-1 had the largest amounts of beta globulin whereas animals infected with 200:NIH had amounts intermediate between HM-1 and the other two strains (table 25). Although the time effect was not significant at the .01 level (table 24), ANOVA suggested a quadratic trend over time. When the highly significant strain effect was partitioned into sums of squares for orthogonal contrasts, HM-1 was related to significantly greater amounts of beta

AD-A128 689 AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY TO ENTAMOEBA HISTOLYTICA(U) JOHNS HOPKINS UNIV BALTIMORE MD R G TAYLOR 15 APR 83 DAMD17-75-C-5001 3/6

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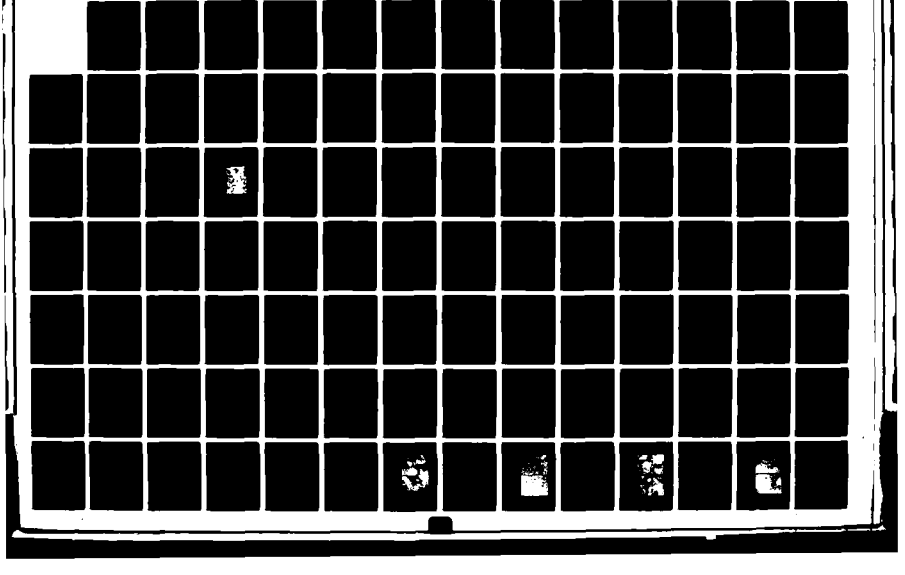
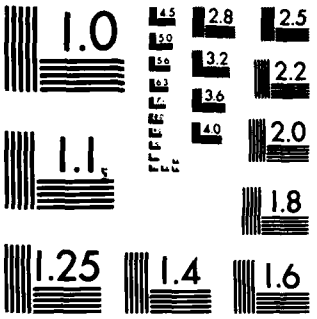




globulin than other ameba strains. Also, 200:NIH had a significantly different effect than HU-21 (table 24). There was no significant difference between HM-1 and 200:NIH or HK-9 by the Duncan procedure (.01 level); HM-1 and 200:NIH were significantly different from HU-21, however.

The most important serum fraction was gamma globulin because of its role in the humoral immune response to infection. Table 26 clearly shows that the strain x time interaction (table 27) was due to HM-1 causing much higher levels of gamma globulin in the serum than the other strains of amebae. A strain x time interaction did not occur among the other three strains. Although the time effect was not significant overall, one cannot ignore the linear trend significant at the .01 level (table 27). Partitioning the sum of squares for strain effect into individual degrees of freedom showed that highly significant variation was due to the difference between HM-1 and the other strains. Although the orthogonal contrasts did not compare HM-1 with 200:NIH directly, the Duncan multiple range test did not detect a significant difference between these two strains at the .01 level. Strain HM-1 caused a dramatic increase in gamma globulin over the period of time covered in this experiment (table 26). Elevated gamma globulin was particularly pronounced at the higher dose levels of HM-1 (figure 63).

In the same graph, one can see that 200:NIH caused elevated gamma globulin only at the highest dose level used. The other two groups were similar to uninfected controls. In table 27 the overall effect of dose was not significant, and control animals did not differ significantly from infected ones. Nevertheless, figure 63 shows clearly that animals infected with HM-1 at all dose levels and 200:NIH at the highest dose level had elevated gamma globulin. Comparison of figures 63 and 61 shows an inverse relationship between the amount of gamma globulin and albumin in the serum of hamsters infected with HM-1 and 200:NIH. This relationship largely explained the results with respect to A:G ratio.

Because antibody resides in the gamma globulin fraction of serum, serology titers for amebiasis were expected to reflect patterns similar to that of gamma globulin. The hemagglutination test proved to be nonspecific with these sera and will not be discussed further. Analysis of variance was done on a $\log_{10}$ transformation of complement fixation titers. The highly significant strain x dose interaction detected by ANOVA (table 28) was due to the much higher geometric mean CF titers on sera from hamsters infected with HM-1 amebae (figure 64). The overall effect of dose was not significant at the .01 level (table 28); but infected animals had significantly higher titers than

uninfected controls, as expected. The overall effect of time was not significant with respect to CF titers, but figure 65 indicates that HM-1 stimulated good antibody production by seven days. Strain 200:NIH was also capable of stimulating CF antibody production, but at least 315 000 200:NIH amebae were required (figure 64). Also, antibody was not detectable in sera from animals infected with 200:NIH until 11 days after infection (figure 65). Strain HM-1 was the most immunogenic strain of axenic E. histolytica used in this experiment and elicited good antibody production as early as seven days after infection.

Comparison of the hamster model for ALA with the human disease revealed only minor differences in serum proteins. Both man and the animal model have normal concentrations of total serum protein but commonly have an inverted A:G ratio resulting from decreased albumin and increased globulin fractions (Powell 1959b). Unlike ALA patients, infected hamsters had normal levels of alpha-1 and alpha-2 globulins. Beta globulin is normal in the human disease but possibly elevated in severe cases of experimental hepatic amebiasis. However, separation of beta and gamma globulin by electrophoresis was difficult when large amounts of gamma globulin were present; elevated beta globulin in hamster serum could represent an artifact. Gamma globulin was markedly elevated

in the model as in man. Rising levels of gamma globulin coincided with increasing titers of complement fixing antibodies first detected in hamster serum seven days after infection. Patients with extra-intestinal amebiasis usually have detectable amounts of antiamebic antibodies in their sera (Stevens et al. 1979). Of the four axenic strains of E. histolytica used in this study, HM-1 most closely reflected pathophysiologic changes in serum proteins that occur in the human disease. Strain 200:NIH caused abnormalities in serum proteins intermediate between HM-1 and control groups.

Liver Function Tests

Several enzymes and total bilirubin were measured on serum samples taken from uninfected controls and animals infected with four strains of axenic amebae.

As expected, total bilirubin did not reflect any significant differences related to amebic infection of the liver. Table 29 shows no discernible trend with time nor any appreciable difference among strains.

Creatine phosphokinase is a serum enzyme commonly elevated in patients with skeletal muscle disease, myocardial infarction, or cerebrovascular accident (Tietz 1976). CPK was measured not because of any hypothesis relating the enzyme to amebiasis but was treated as a

dummy variable designed to test whether analysis of variance would reveal spurious variation in the experimental design. None was found.

The transaminases and lactate dehydrogenase all exhibited a similar pattern. In each case, HM-1 caused elevated enzyme levels soon after surgery that declined to the same levels as the other strains by 11 days (figures 66, 67, and 68). Strain 200:NIH caused a slight elevation of aspartate and alanine aminotransferases and lactate dehydrogenase 11 days after infection. Analysis of variance in tables 30, 31, and 32 did not detect a significant dose effect or a significant difference between controls and infected animals. Thus it is unlikely that AST, ALT, or LDH responded to the effects of amebic infection. One cannot ignore the interaction of HM-1 with the time of infection early in the disease when necrosis due to amebae was minimal. Although a significant time effect did not occur with these three enzymes, HM-1 produced significantly higher levels than the other strains (tables 30-32).

Alkaline phosphatase often reaches high levels in the serum of patients with amebic liver abscess and was expected to be elevated in the serum of hamsters with severe necrosis of the liver caused by Entamoeba histolytica. In table 33, the strain x time interaction was not statistically

significant; but figure 69 shows that HM-1 was indeed responsible for a strain x time interaction, because it behaved in a different manner than the other strains. Alkaline phosphatase rose to extremely high levels by seven days after infection in hamsters infected with HM-1; the amount of ALP in the serum of these hamsters seemed to become asymptotic above 5000 mU/ml (figure 69). The effect of time was not statistically significant at the .01 level, but ANOVA in table 33 suggested a nonlinear trend evident in the HM-1 curve of figure 69. The dose effect was due to a highly significant difference between controls and infected animals. Figure 70 shows that the level of alkaline phosphatase in serum was related to the dose of HM-1 amebae, although the smallest dose caused much higher levels of ALP than in control animals or those infected with other strains of amebae. Animals infected with 200:NIH had slightly elevated ALP only at the higher dose levels (figure 70) but not at the end of the experiment (figure 69). Alkaline phosphatase in hamsters infected with HU-21 and HK-9 remained at control levels.

With respect to dose, aldolase had results similar to alkaline phosphatase. In figure 71 one can see that increasing numbers of HM-1 amebae produced higher levels of aldolase in the serum. However, 200:NIH caused elevation of

ALD only at the highest dose used. The other strains of amebae did not cause increased amounts of aldolase above control levels. Figure 72 illustrates very nicely the absence of interaction between strain and time (table 34). The effect of HM-1 was additive above the levels of the other strains and controls at each time interval. ANOVA (table 34) confirmed that a highly significant strain effect was due to much higher levels of aldolase in the HM-1 group compared to other strains. The most significant finding with regard to aldolase was that experimentally infected hamsters had significantly elevated levels of this enzyme before alkaline phosphatase became elevated but in the same animals that developed severe necrosis of the liver due to amebic infection later on. In addition, levels of aldolase and alkaline phosphatase were related to the number of HM-1 amebae in those hamsters that developed severe liver disease.

Comparing liver function tests in ALA patients with the hamster model revealed close similarities. Liver function tests other than bilirubin may be abnormal in amebic liver abscess, but the changes are not specific for amebiasis (Powell 1959a). Alkaline phosphatase is more likely to be elevated than transaminases. Bilirubin was normal in the model. In the HM-1 group lactate dehydrogenase and the

transaminases were elevated soon after surgery but declined to normal levels within 11 days, paradoxically when the most destructive tissue necrosis was setting in. Alkaline phosphatase was a reliable indicator of liver damage because it reached extremely high levels in animals with the most severe disease. Aldolase became elevated sooner than ALP, and both enzymes demonstrated a dose response to the number of HM-1 amebae.

Summary of Pathophysiology

Axenic strain HM-1 of E. histolytica produced pathophysiological changes in hamsters reminiscent of amebic liver abscess in man. Weight loss can be part of the clinical picture for the human disease (Ochsner and DeBakey 1943, Barbour and Juniper 1972). Initial loss of body weight in hamsters resulted from surgical trauma. Unlike sham-operated controls and animals inoculated with three other axenic strains of E. histolytica, the HM-1 group with severe disease suffered persistent weight loss after accounting for enlargement of the liver. Animals with severe liver disease caused by HM-1 had anemia and neutrophilic leukocytosis, the same blood picture seen in ALA patients (Ochsner and DeBakey 1943). In the hamster model, HM-1 was the only amebic strain associated with basophils in the peripheral blood. As with sera from ALA patients

(Powell 1959b), serum protein electrophoresis on hamster sera from the HM-1 group showed an inverted A:G ratio caused by less albumin and more globulins than normal. Markedly elevated *gamma* globulin in hamsters coincided with rising CF titers beginning seven days after infection. These findings agreed with the human disease. Two differences were noted when comparing electrophoretic patterns from man and the hamster. Alpha-1 and alpha-2 globulins are often elevated in ALA patients but not in the animal model. Beta globulin is normal in man but elevated in hamsters. However, large amounts of gamma globulin in hamster sera could cause artificially high beta globulin peaks. Liver function tests performed on hamsters that received HM-1 were similar to results from ALA patients. Bilirubin was normal, as expected (Powell 1959b). Although LDH and aminotransferases were elevated early during HM-1 infection, levels of these enzymes were inversely proportional to the degree of liver damage. Alkaline phosphatase was directly proportional to the amount of tissue necrosis, but aldolase became elevated sooner than ALP in the course of severe liver disease. Patients with amebic liver abscess commonly have elevated ALP (Sepúlveda et al. 1959, Salako 1967, Kamat et al. 1968, Barbour and Juniper 1972) and AST (Sheehy et al. 1968,

Santhanagopalan et al. 1968) but less frequently, elevated ALT (Santhanagopalan et al. 1968).

Summary of Strain x Time x Dose Experiment

This study established the validity of inbred LHC/Lak hamsters as a model for amebic liver abscess. Observations on the pathology and pathophysiology of hepatic amebiasis in this model were similar to descriptions of the same disease in man.

Of four axenic strains of Entamoeba histolytica, HM-1 best mimicked the human disease. Table 35 summarizes the general pathology and pathophysiology of hepatic amebiasis produced by this strain following intraportal inoculation. The pathophysiologic changes in hamsters coincided to a remarkable degree with similar changes known to occur in patients with amebic liver abscess. For example, the animals had persistent weight loss after accounting for hepatomegaly. They had splenomegaly, anemia, and leukocytosis due to increased numbers of neutrophils. An inverted A:G ratio resulted from decreased albumin and elevated globulins in serum. Increased gamma globulin was correlated with complement fixing antibodies as serum levels of alkaline phosphatase became proportional to the amount of necrosis in the liver. Although the pathogenesis of amebic liver abscess is incompletely understood, findings in tissue

sections from hamsters indicated that the same necrotic process held true in the model. Unlike man, experimentally infected hamsters responded with a granulomatous reaction to amebic invasion of the liver; but this type of tissue reaction may develop later into an histological picture more typical for ALA.

Strain 200:NIH was intermediate in pathogenicity between HM-1, a virulent parasite in the liver of hamsters, and HU-21 or HK-9, essentially nonpathogenic strains. The effects of 200:NIH amebae in the hamster model are summarized in table 36. In general, strain 200:NIH produced similar but less dramatic pathological changes than HM-1 until the experimental infection progressed beyond 11 days. As an exception, the leukocytosis with 200:NIH was greater than with HM-1. As early as seven days after infection with 200:NIH, signs of healing began to appear in the liver; and no active amebic lesions with viable amebae were seen at 15 days. In the 200:NIH group necropsied then, both the liver and spleen were normal size. The blood picture, serum protein electrophoresis, serology, and liver function tests were all normal or at considerably lower levels than previous time intervals in the experiment. All these findings were consistent 15 days after infection with 200:NIH when the liver was undergoing repair. Because

experimental conditions for 200:NIH and HM-1 groups were the same except for the strain of organism, these results suggested that the two amebic strains have one or more determinants of pathogenicity by which they differ.

TABLE 14

ANALYSIS OF VARIANCE FOR CHANGE IN BODY WEIGHT AT DIFFERENT LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	46.63066	3	15.54355	.78474	.513
HM-1 vs other strains	5.67189	1	5.67189	.28635	.597
HK-9 vs HU-21 + 200:NIH	.68345	1	.68345	.03450	.854
200:NIH vs HU-21	40.27533	1	40.27533	2.03335	.165
Time (T)	531.60804	3	177.20268	8.94631	<.0005
Linear	467.06107	1	467.06107	23.58020	<.0005
Quadratic	24.50251	1	24.50251	1.23704	.276
Cubic	40.04447	1	40.04447	2.02170	.167
Dose (D)	63.71314	3	21.23771	1.07221	.377
Control vs infected	16.45020	1	16.45020	.83051	.370
Linear	40.27533	1	40.27533	2.03335	.165
Quadratic	6.98761	1	6.98761	.35278	.557
S x T interaction	151.90567	9	16.87841	.85213	.577
S x D interaction	218.58560	9	24.28729	1.22618	.320
T x D interaction	279.33320	9	31.03702	1.56695	.176
Error	534.79830	27	19.80734		

Figure 50. Change in body weight of hamsters from preinfection to time of necropsy.

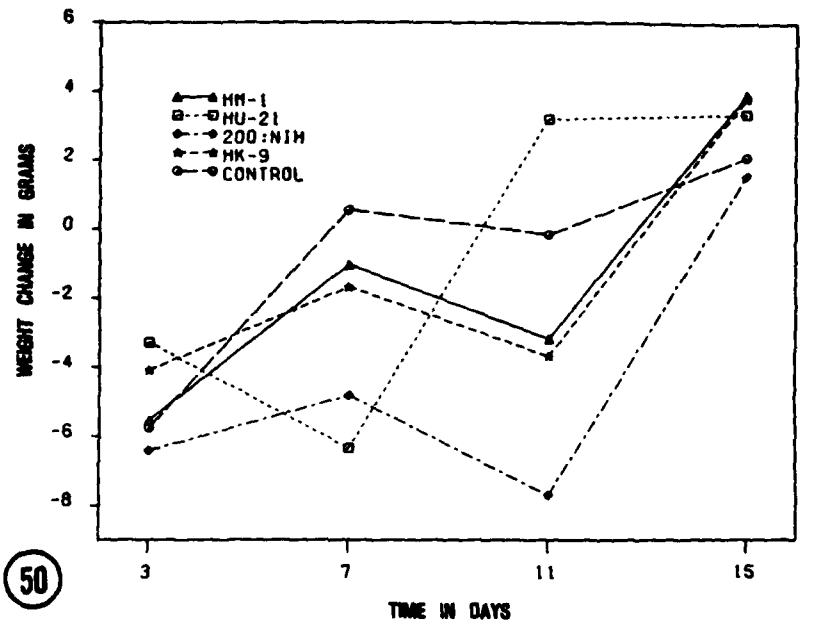

6
4
2
0
-2
-4
-6
-8
WEIGHT CHANGE IN GRAMS
HM-1
MU-21
200:NIH
HK-9
CONTROL
3
7
11
15
TIME IN DAYS
50

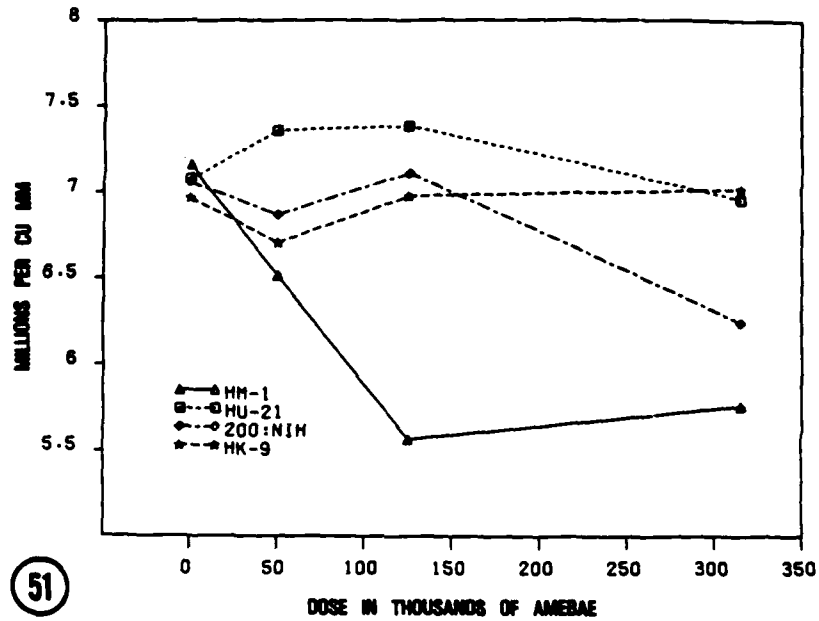
TABLE 15

ANALYSIS OF VARIANCE IN ERYTHROCYTE COUNT AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

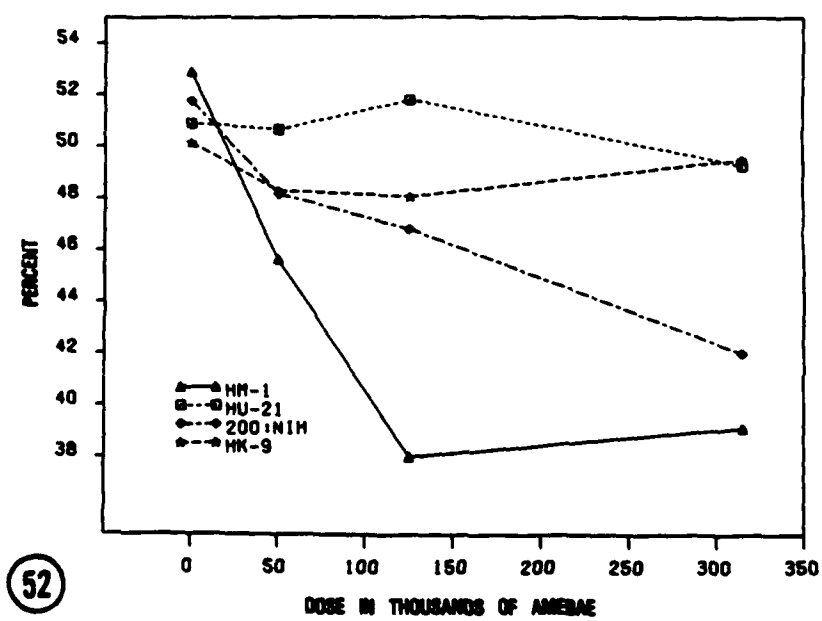
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	7.55206	3	2.51735	10.85051	<.0005
HM-1 vs other strains	6.34380	1	6.34380	27.34361	<.0005
HK-9 vs HU-21 + 200:NIH	.08700	1	.08700	.37500	.545
200:NIH vs HU-21	1.12125	1	1.12125	4.83292	.037
Time (T)	3.57033	3	1.19011	5.12972	.006
Linear	2.20448	1	2.20448	9.50195	.005
Quadratic	.10081	1	.10081	.43450	.515
Cubic	1.26504	1	1.26504	5.45271	.027
Dose (D)	2.68483	3	.89494	3.85746	.020
Control vs infected	1.54442	1	1.54442	6.65689	.016
Linear	1.06945	1	1.06945	4.60965	.041
Quadratic	.07096	1	.07096	.30586	.585
S x T interaction	3.54106	9	.39345	1.69589	.139
S x D interaction	6.38441	9	.70938	3.05763	.012
T x D interaction	3.37293	9	.37477	1.61537	.161
Error	6.26408	27	.23200		

Figure 51. Relationship of RBC count to dose of amebae. Strain HM-1 caused pronounced anemia at higher dose levels whereas 200:NIH did so only at the highest dose.

Figure 52. Microhematocrit as a function of inoculum size. Hamsters that received at least 125 thousand HM-1 or 315 thousand 200:NIH amebae were mildly anemic. Compare figure 51.


8
7.5
7
6.5
6
5.5
MILLIONS PER CU MM
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

51


54
52
50
48
46
44
42
40
38
PERCENT
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

52

TABLE 16

ANALYSIS OF VARIANCE IN MICROHEMATOCRIT AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	397.57422	3	132.52474	8.04966	.001
HM-1 vs other strains	301.25130	1	301.25130	18.29825	<.0005
HK-9 vs HU-21 + 200:NIH	.06510	1	.06510	.00395	.950
200:NIH vs HU-21	96.25781	1	96.25781	5.84678	.023
Time (T)	295.98047	3	98.25781	5.99270	.003
Linear	245.87578	1	245.87578	14.93469	.001
Quadratic	2.44141	1	2.44141	.14829	.703
Cubic	47.66328	1	47.66328	2.89511	.100
Dose (D)	382.07422	3	127.35807	7.73583	.001
Control vs infected	298.75130	1	298.75130	18.14639	<.0005
Linear	81.28125	1	81.28125	4.93709	.035
Quadratic	2.04167	1	2.04167	.12401	.727
S x T interaction	273.03516	9	30.33724	1.84271	.106
S x D interaction	402.81641	9	44.75738	2.71860	.021
T x D interaction	222.53516	9	24.72613	1.50188	.198
Error	444.51172	27	16.46340		

TABLE 17

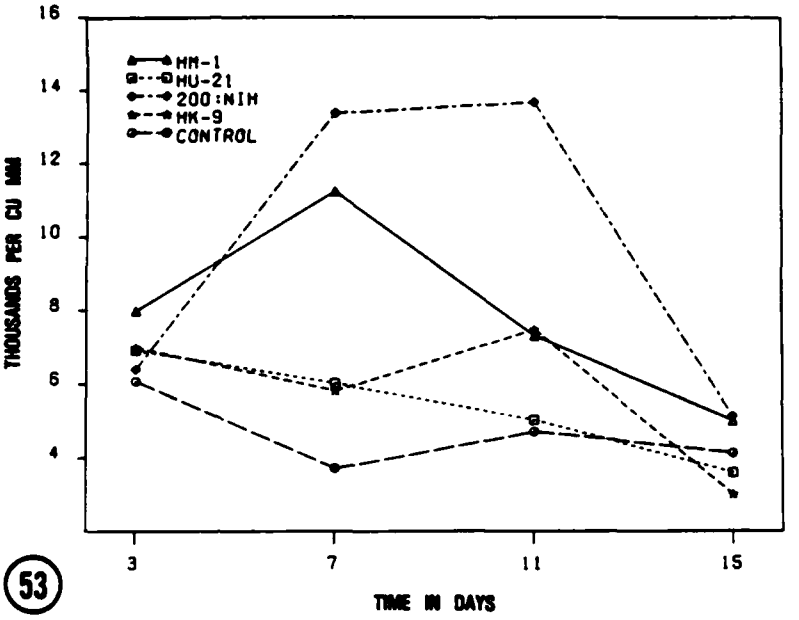
ANALYSIS OF VARIANCE IN LEUKOCYTE COUNT AT FOUR LEVELS OF STRAIN, TIME, AND DOSE

WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

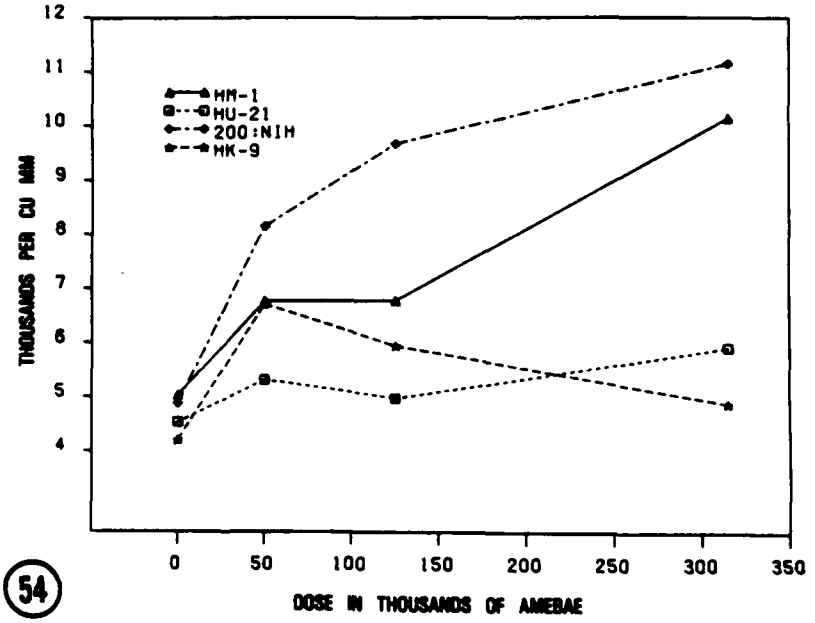
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	114742868	3	338247622	4.38860	.012
HM-1 vs other strains	8120542	1	8120542	.93177	.343
HK-9 vs HU-21 + 200:NIH	20715771	1	20715771	2.37697	.135
200:NIH vs HU-21	85906555	1	85906555	9.85707	.004
Time (T)	129646880	3	43215626	4.95864	.007
Linear	55022396	1	55022396	6.31337	.018
Quadratic	72165025	1	72165025	8.28035	.008
Cubic	2459459	1	2459459	.28220	.600
Dose (D)	93064162	3	31021387	3.55945	.027
Control vs infected	76747563	1	76747563	8.80615	.006
Linear	13177694	1	13177694	1.51203	.229
Quadratic	3138905	1	3138905	.36016	.553
S x T interaction	80861924	9	8984658	1.03092	.442
S x D interaction	66904717	9	7433857	.85297	.576
T x D interaction	183180233	9	20353359	2.33538	.043
Error	235310904	27	8715218		

Figure 53. Temporal changes in WBC count. Leukocytosis was pronounced in HM-1 and 200:NIH groups at seven and 11 days but not at the end of the experiment.

Figure 54. Relationship of WBC count to inoculum size. Dose-related leukocytosis was greater in the 200:NIH group than in the HM-1 group.


HM-1
HU-21
200:NIH
HK-9
CONTROL
THOUSANDS PER CU MM
16
14
12
10
8
6
4
3
7
11
15
TIME IN DAYS

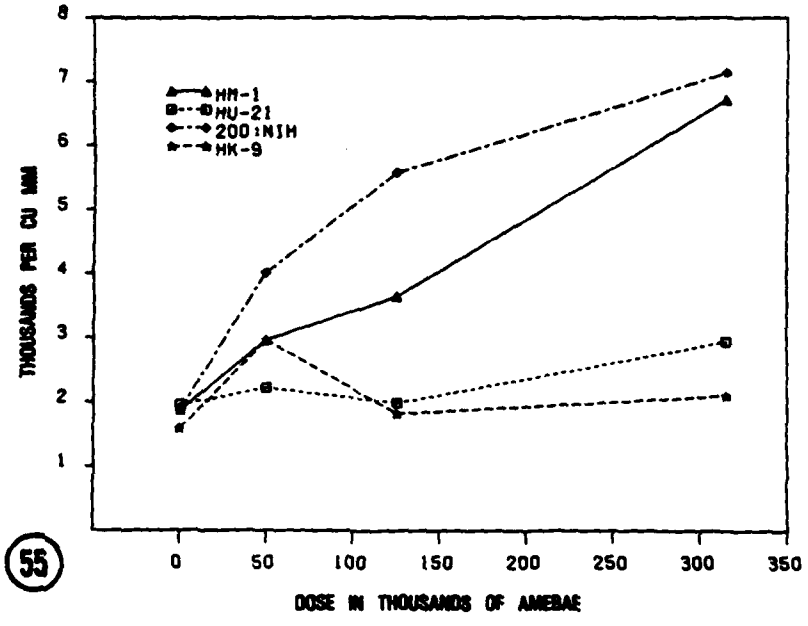
53


HM-1
HU-21
200:NIH
HK-9
THOUSANDS PER CU MM
12
11
10
9
8
7
6
5
4
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

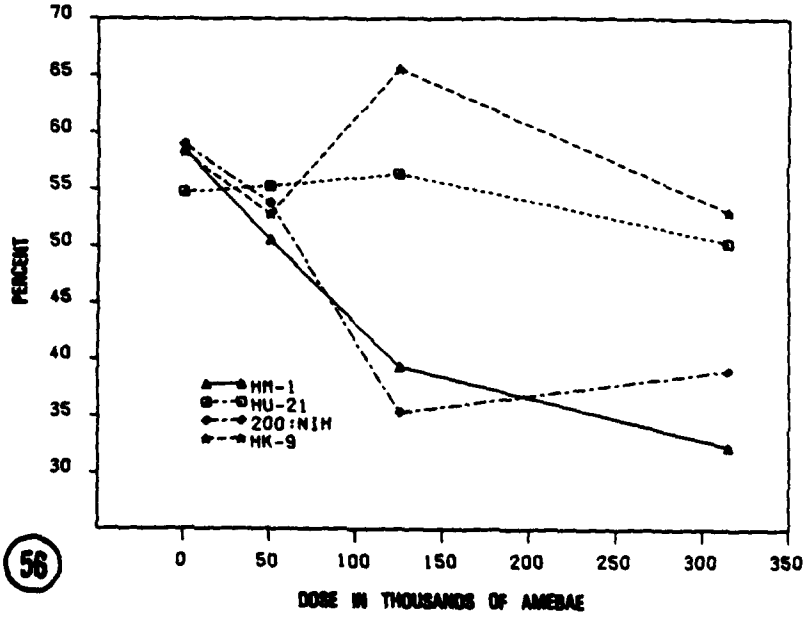
54

Figure 55. Relationship of dose with the actual number of neutrophils determined from differential blood counts and total WBC counts. Compare figure 54.

Figure 56. Percentage of peripheral lymphocytes related to dose of amebae. Groups HM-1 and 200:NIH at higher dose levels had proportionately fewer lymphocytes.


THOUSANDS PER CU MM
8
7
6
5
4
3
2
1
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

55


PERCENT
70
65
60
55
50
45
40
35
30
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

56

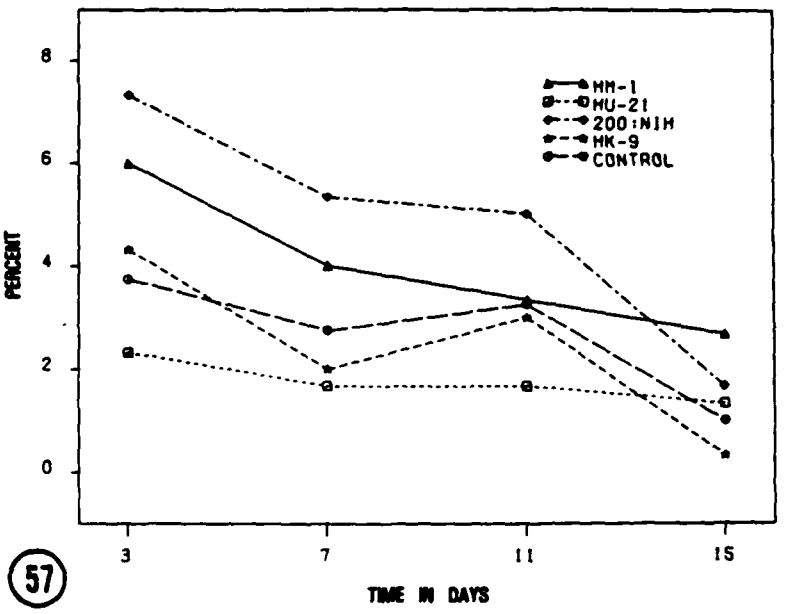
TABLE 18

ANALYSIS OF VARIANCE IN PERCENTAGE OF MONOCYTES AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

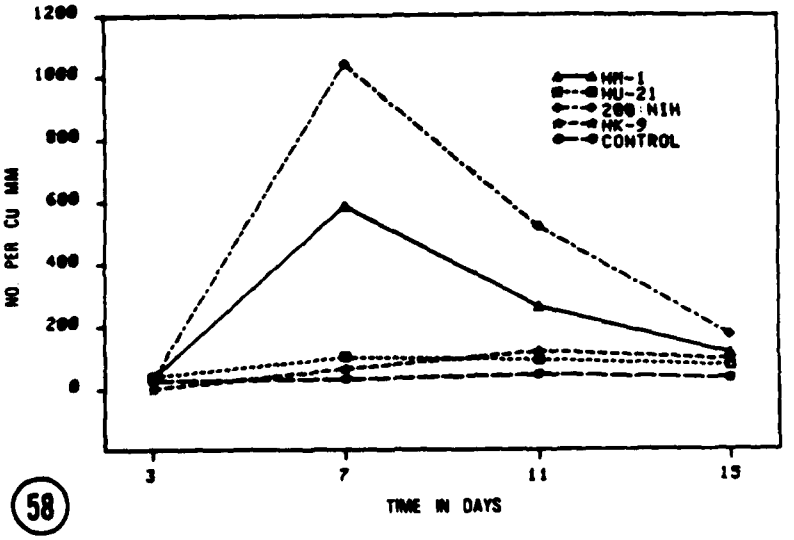
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	53.04687	3	17.68229	3.50925	.029
HM-1 vs other strains	4.38021	1	4.38021	.86930	.359
HK-9 vs HU-21 + 200:NIH	8.16667	1	8.16667	1.62076	.214
200:NIH vs HU-21	40.50000	1	40.50000	8.03767	.009
Time (T)	88.29687	3	29.43229	5.84116	.003
Linear	77.02812	1	77.02812	15.28708	.001
Quadratic	.39062	1	.39062	.07752	.783
Cubic	10.87812	1	10.87812	2.15888	.153
Dose (D)	6.92187	3	2.30729	.45791	.714
Control vs infected	3.79687	1	3.79687	.75353	.393
Linear	.78125	1	.78215	.15505	.697
Quadratic	2.34375	1	2.34375	.46514	.501
S x T interaction	11.14062	9	1.23785	.24566	.984
S x D interaction	36.01562	9	4.00174	.79419	.624
T x D interaction	72.76562	9	8.08507	1.60457	.164
Error	136.04687	27	5.03877		

Figure 57. Temporal changes in the percentage of peripheral monocytes.

Figure 58. Temporal changes in the actual number of peripheral eosinophils. Strains HM-1 and 200:NIH elicited eosinophilia in hamsters bled seven and 11 days after infection.


8
6
4
2
0
PERCENT
MM-1
MU-21
200:NIH
MK-9
CONTROL
3
7
11
15
TIME IN DAYS

57


1200
1000
800
600
400
200
0
NO PER CU MM
MM-1
MU-21
200:NIH
MK-9
CONTROL
3
7
11
15
TIME IN DAYS

58

TABLE 19

ANALYSIS OF VARIANCE IN NUMBER OF PERIPHERAL EOSINOPHILS AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	841755.88	3	280585.29	4.45523	.011
HM-1 vs other strains	20422.42	1	20422.42	.32427	.574
HK-9 vs HU-21 + 200:NIH	207249.61	1	207249.61	3.29078	.081
200:NIH vs HU-21	614083.86	1	614083.86	9.75064	.004
Time (T)	879289.70	3	293096.57	4.65389	.010
Linear	1.10	1	1.10	.00002	.997
Quadratic	652698.07	1	652698.07	10.36377	.003
Cubic	226590.53	1	226590.53	3.59788	.069
Dose (D)	529377.63	3	276459.21	2.80188	.059
Control vs infected	296889.44	1	296889.44	4.71412	.039
Linear	23073.27	1	23073.27	.36637	.550
Quadratic	209414.92	1	209414.92	3.32516	.079
S x T interaction	939281.70	9	104364.63	1.65714	.149
S x D interaction	1057260.21	9	117473.36	1.85428	.102
T x D interaction	860422.06	9	95602.45	1.51801	.192
Error	1700428.29	27	62978.83		

Figure 59. Basophil in the blood of a hamster with hepatic amebiasis caused by axenic strain HM-1.

59

TABLE 20

ANALYSIS OF VARIANCE IN A:G OF SERUM AT FOUR LEVELS OF STRAIN, TIME, AND DOSE

WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	2.95870	3	.98623	10.84962	<.0005
HM-1 vs other strains	2.52289	1	2.52289	27.75449	<.0005
HK-9 vs HU-21 + 200:NIH	.09632	1	.09632	1.05963	.312
200:NIH vs HU-21	.33949	1	.33949	3.73473	.064
Time (T)	1.10839	3	.36946	4.08447	.017
Linear	.35980	1	.35980	3.95821	.057
Quadratic	.52548	1	.52548	5.78079	.023
Cubic	.22311	1	.22311	2.45441	.129
Dose (D)	1.73216	3	.57739	6.35185	.002
Control vs infected	1.62171	1	1.62171	17.84050	<.0005
Linear	.09943	1	.09943	1.09385	.305
Quadratic	.01102	1	.01102	.12120	.730
S x T interaction	1.84635	9	.20515	2.25686	.049
S x D interaction	2.02271	9	.22475	2.47243	.033
T x D interaction	.57688	9	.06410	.70514	.699
Error	2.45431	27	.09090		

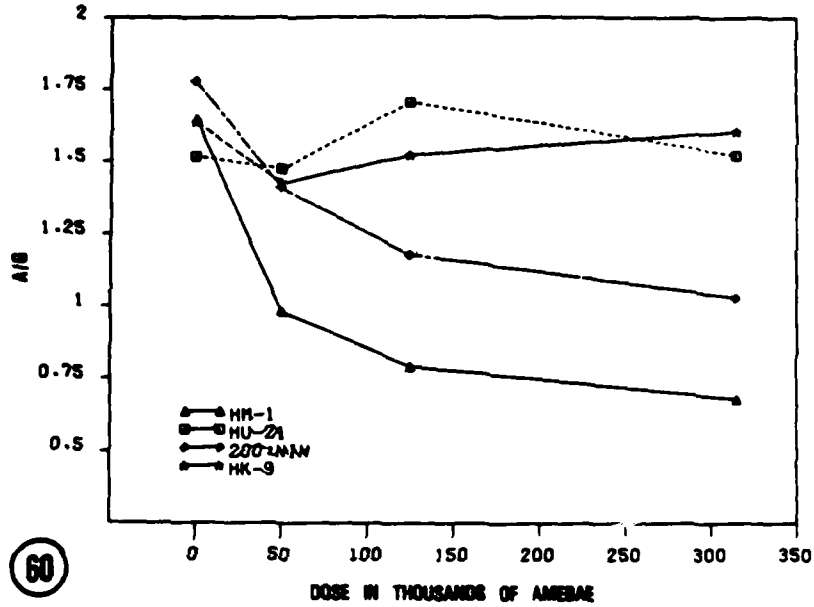
TABLE 21

A:G IN SERUM FROM HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INOCULATION

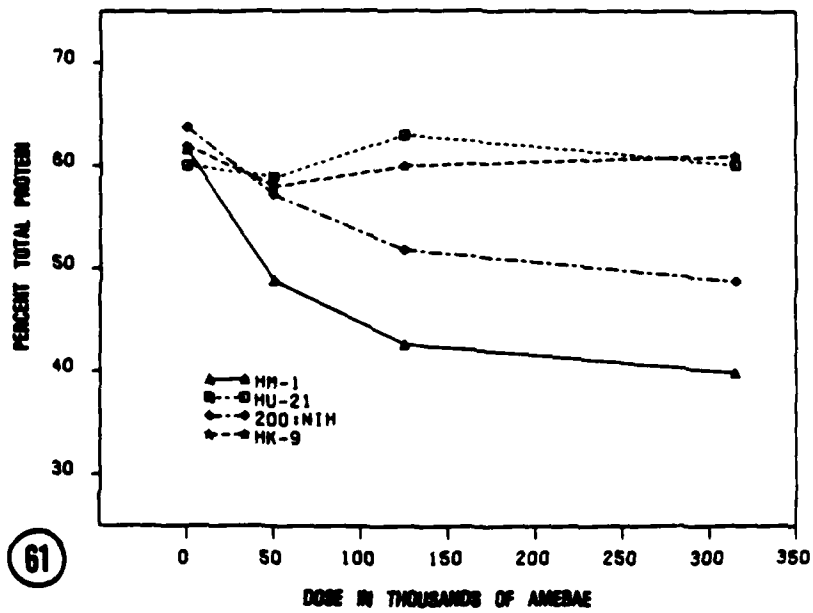
Time after inoculation	Strain				Uninfected Controls
	HM-1	HU-21	200:NIH	HK-9	
3 days	.998	1.646	1.142	1.238	1.553
7 days	.771	1.390	1.117	1.550	1.666
11 days	.789	1.542	.712	1.290	1.671
15 days	.704	1.683	1.842	1.980	1.679
Mean	.8156	1.5652	1.2029	1.5147	1.6422

Figure 60. Albumin to globulin ratio as a function of dose. A:G was dose related in HM-1 and 200:NIH groups, but only HM-1 consistently caused an inverted A:G ratio.

Figure 61. Serum albumin related to dose of amebae. Strains HM-1 and 200:NIH depressed serum albumin, but 200:NIH did so only at higher dose levels.


2
1.75
1.5
1.25
1
0.75
0.5
A/G
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

60


70
60
50
40
30
PERCENT TOTAL PROTEIN
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

61

TABLE 22

ANALYSIS OF VARIANCE IN ALBUMIN AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	*F*	*p*
Strain (S)	1579.08541	3	526.36180	14.52733	<.0005
HM-1 vs other strains	1315.13640	1	1315.13640	36.29712	<.0005
HK-9 vs HU-21 + 200:NIH	55.35841	1	55.35841	1.52786	.227
200:NIH vs HU-21	208.59061	1	208.59061	5.75700	.024
Time (T)	249.30437	3	83.10146	2.29356	.101
Linear	31.68904	1	31.68904	.87460	.358
Quadratic	154.69150	1	154.69150	4.26941	.049
Cubic	62.92383	1	62.92383	1.73667	.199
Dose (D)	798.60300	3	266.20100	7.34702	.001
Control vs infected	709.55644	1	709.55644	19.58341	<.0005
Linear	88.44489	1	88.44489	2.44104	.130
Quadratic	.60167	1	.60167	.01661	.898
S x T interaction	874.39626	9	97.15514	2.68143	.023
S x D interaction	924.92752	9	102.76972	2.83639	.017
T x D interaction	238.28889	9	26.47654	.73074	.677
Error	978.27825	27	36.23253		

TABLE 23

SERUM ALBUMIN FROM HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INOCULATION

Time after inoculation	Strain				Uninfected Controls
	HM-1	HU-21	200:NIH	HK-9	
3 days	49.70*	61.43	53.00	55.27	60.20
7 days	43.53	57.77	50.83	60.67	62.32
11 days	44.07	60.57	41.53	56.03	62.15
15 days	37.53	62.57	64.70	66.37	62.48
Mean	43.708	60.583	52.517	59.583	61.788

* Percentage of total protein.

Figure 62. Serum protein electrophoresis. A. Normal hamster. B. Hamster infected with HM-1 trophozoites 15 days previously.

(62)

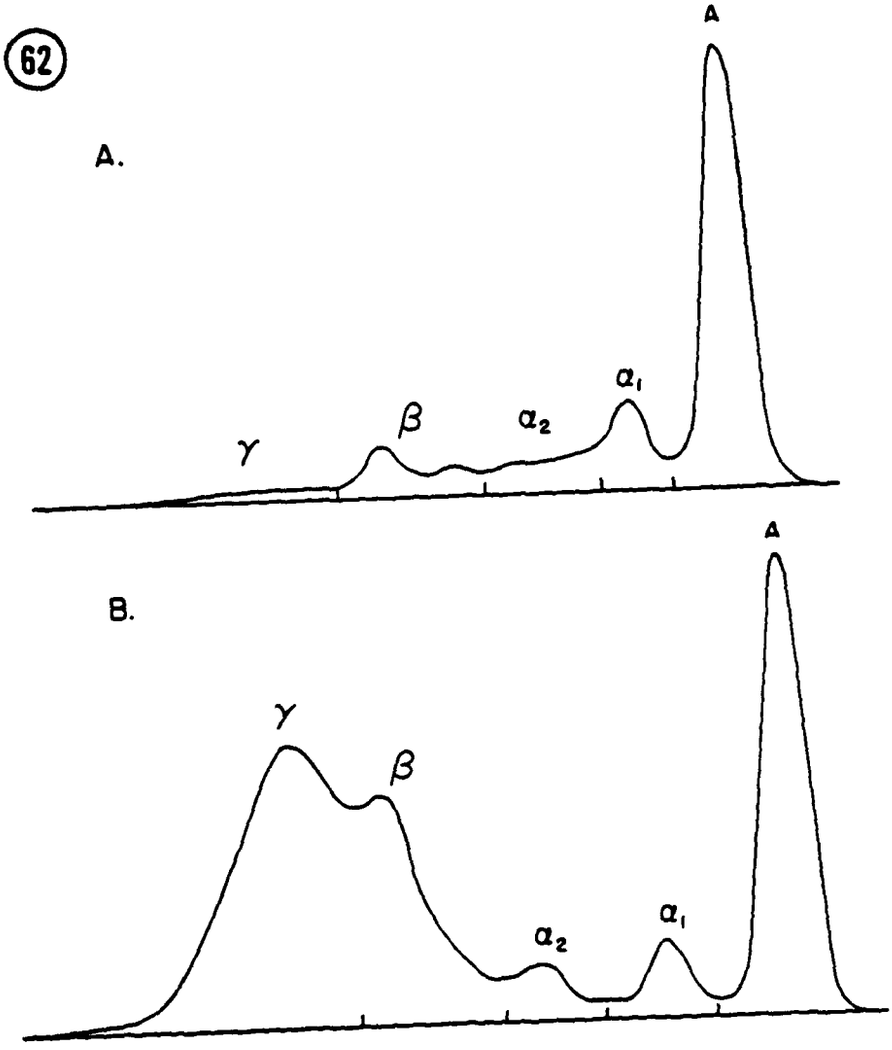


TABLE 24

ANALYSIS OF VARIANCE IN BETA GLOBULIN AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	222.14012	3	74.04671	7.36762	.001
HM-1 vs other strains	135.84480	1	135.84480	13.51651	.001
HK-9 vs HU-21 + 200:NIH	3.41261	1	3.41261	.33955	.565
200:NIH vs HU-21	82.88271	1	82.88271	8.24680	.008
Time (T)	118.38793	3	39.46264	3.92652	.019
Linear	33.60528	1	33.60528	3.34371	.079
Quadratic	69.51390	1	69.51390	6.91661	.014
Cubic	15.26875	1	15.26875	1.51924	.228
Dose (D)	147.98667	3	49.32889	4.90821	.008
Control vs infected	146.47542	1	146.47542	14.57426	.001
Linear	.22781	1	.22781	.02267	.881
Quadratic	1.28344	1	1.28344	.12770	.724
S x T interaction	56.13392	9	6.23710	.62059	.769
S x D interaction	239.80506	9	26.64501	2.65117	.024
T x D interaction	175.44747	9	19.49416	1.93966	.089
Error	271.35767	27	10.05028		

TABLE 25

BETA GLOBULIN IN SERUM FROM HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AT DOSE LEVELS AVERAGED OVER TIME AFTER INFECTION

Dose in Thousands	Strain			
	HM-1	HU-21	200:NIH	HK-9
Control*	14.18**	15.00	13.28	15.28
50	20.55	13.35	17.52	20.15
125	20.40	14.58	20.75	16.90
315	23.18	14.72	18.98	14.02
Mean	21.377	14.217	19.083	17.023

* Overall control mean = 14.431.

** Percentage of total protein.

TABLE 26

GAMMA GLOBULIN IN HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INOCULATION

Time after inoculation	Strain				Uninfected Controls
	HM-1	HU-21	200:NIH	HK-9	
3 days	3.67*	3.87	3.77	3.53	3.62
7 days	11.10	6.37	5.97	5.37	4.42
11 days	14.17	4.43	16.67	4.17	4.50
15 days	29.00	4.57	4.77	3.43	4.40
Mean	14.483	4.808	7.792	4.125	4.238

* Percentage of total protein.

TABLE 27

ANALYSIS OF VARIANCE IN GAMMA GLOBULIN AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	547.92772	3	182.64257	5.51036	.004
HM-1 vs other strains	507.32482	1	507.32482	15.30608	.001
HK-9 vs HU-21 + 200:NIH	15.04166	1	15.04166	.45381	.506
200:NIH vs HU-21	25.56124	1	25.56124	.77119	.388
Time (T)	275.53661	3	91.84554	2.77100	.061
Linear	251.87240	1	251.87240	7.59903	.010
Quadratic	23.16018	1	23.16018	.69875	.411
Cubic	.50403	1	.50403	.01521	.903
Dose (D)	288.04658	3	96.01553	2.89680	.053
Control vs infected	152.47500	1	152.47500	4.60020	.041
Linear	123.24492	1	123.24492	3.71832	.064
Quadratic	12.32666	1	12.32666	.37190	.547
S x T interaction	742.88467	9	82.54274	2.49033	.032
S x D interaction	481.09496	9	53.45500	1.61275	.162
T x D interaction	199.96630	9	22.21848	.67034	.728
Error	894.92367	27	33.14552		

Figure 63. Relationship of gamma globulin to inoculum size. Both HM-1 and 200:NIH groups had elevated gamma globulin but the latter, only at the highest dose level. Compare figures 60 and 61.

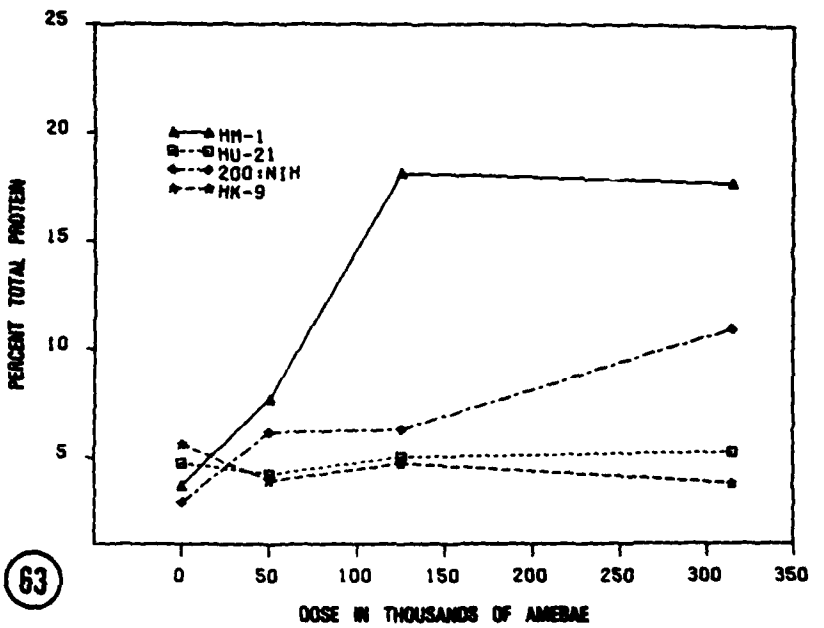

25
20
15
10
5
PERCENT TOTAL PROTEIN
HM-1
HU-21
200:NIH
HK-9
63
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

TABLE 28

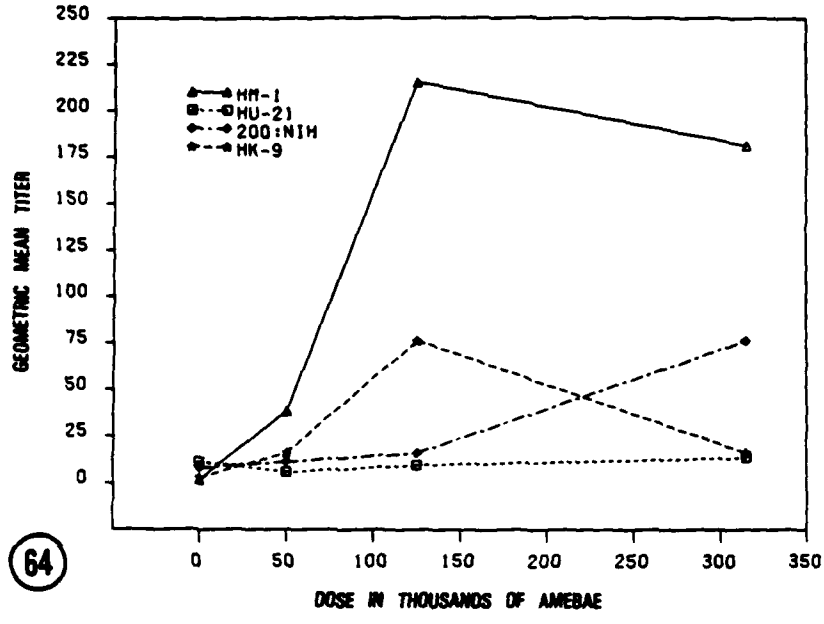
ANALYSIS OF VARIANCE IN COMPLEMENT FIXATION TITERS* AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	1.24226	3	.41409	7.70159	.001
HM-1 vs other strains	1.03004	1	1.03004	19.15768	<.0005
HK-9 vs HU-21 + 200:NIH	.00680	1	.00680	.12639	.725
200:NIH vs HU-21	.20542	1	.20542	3.82068	.061
Time (T)	.26381	3	.08794	1.63551	.204
Linear	.01220	1	.01220	.22682	.638
Quadratic	.01169	1	.01169	.21742	.645
Cubic	.23992	1	.23992	4.46229	.044
Dose (D)	.53388	3	.17796	3.30989	.035
Control vs infected	.45378	1	.45378	8.43984	.007
Linear	.06632	1	.06632	1.23344	.277
Quadratic	.01379	1	.01379	.25640	.617
S x T interaction	.21273	9	.02364	.43963	.901
S x D interaction	1.80587	9	.20065	3.73192	.004
T x D interaction	.44055	9	.04895	.91042	.531
Error	1.45170	27	.05377		

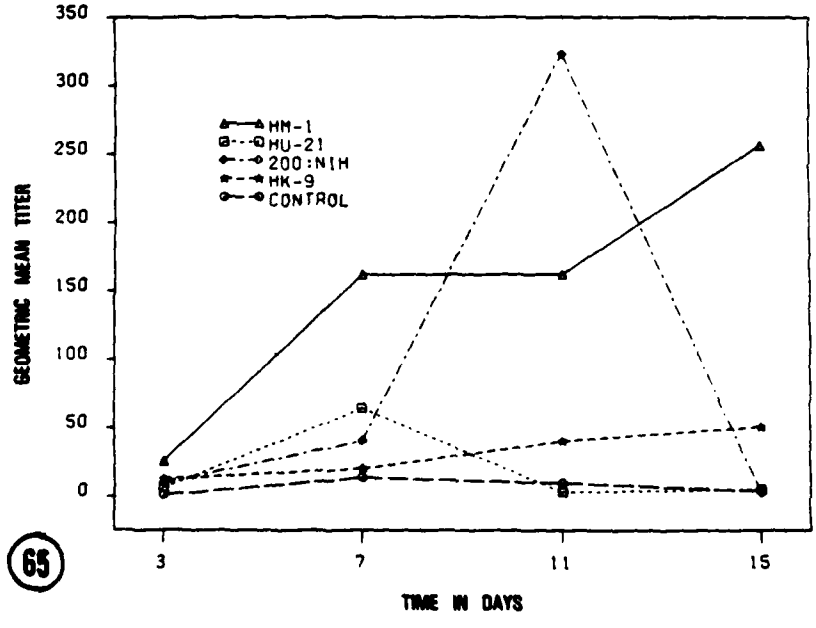
* Log_{10} transformation.

Figure 64. Complement fixing antibodies related to dose of amebae. At least 125 thousand HM-1 trophozoites were required to elicit high antibody titers. Compare figure 63.

Figure 65. Production of complement fixing antibodies during the course of experimental hepatic amebiasis in the hamster. Strain HM-1 stimulated good antibody production by seven days.


250
225
200
175
150
125
100
75
50
25
0
GEOMETRIC MEAN TITER
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

64


350
300
250
200
150
100
50
0
GEOMETRIC MEAN TITER
HM-1
HU-21
200:NIH
HK-9
CONTROL
3
7
11
15
TIME IN DAYS

65

TABLE 29

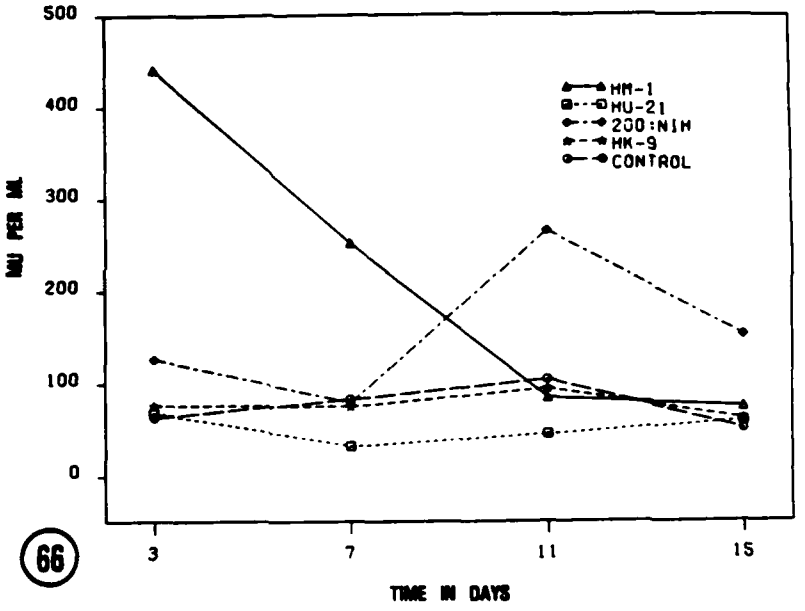
TOTAL BILIRUBIN IN SERUM FROM HAMSTERS INFECTED WITH FOUR STRAINS OF AMEBAE AVERAGED OVER DOSE LEVELS WITH NECROPSY AT INTERVALS AFTER INFECTION

Time after inoculation	Strain				Uninfected Controls
	HM-1	HU-21	200:NIH	HK-9	
3 days	.20*	.23	.23	.47	.48
7 days	.37	.43	.40	.40	.28
11 days	.30	.40	.43	.50	.45
15 days	.47	.43	.47	.50	.50
Mean	.333	.375	.383	.467	.425

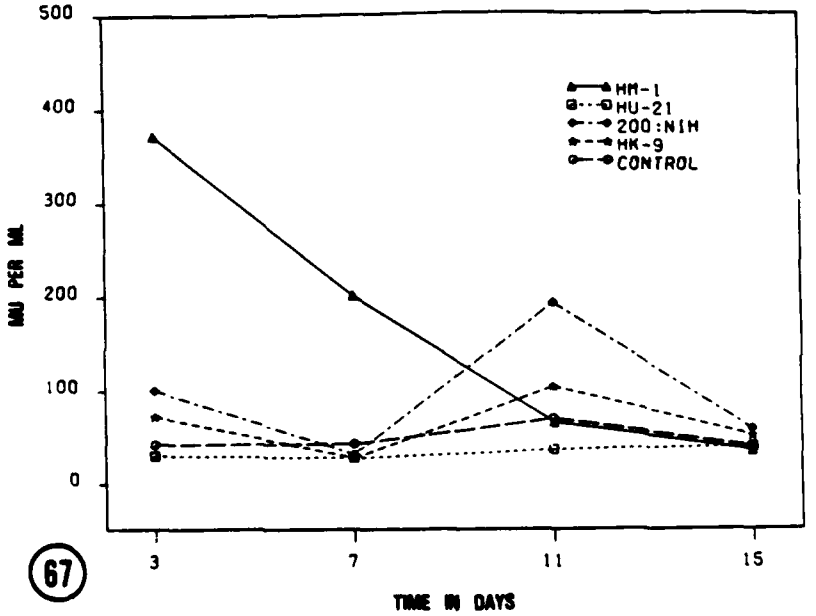
* mg/100 ml.

Figure 66. Temporal changes in serum aspartate aminotransferase.

Figure 67. Temporal changes in serum alanine aminotransferase.


500
400
300
200
100
0
MU PER ML
HM-1
HU-21
200:NIH
HK-9
CONTROL
3
7
11
15
TIME IN DAYS

66


500
400
300
200
100
0
MU PER ML
HM-1
HU-21
200:NIH
HK-9
CONTROL
3
7
11
15
TIME IN DAYS

67

Figure 68. Temporal changes in serum lactate dehydrogenase. Compare figures 66 and 67.

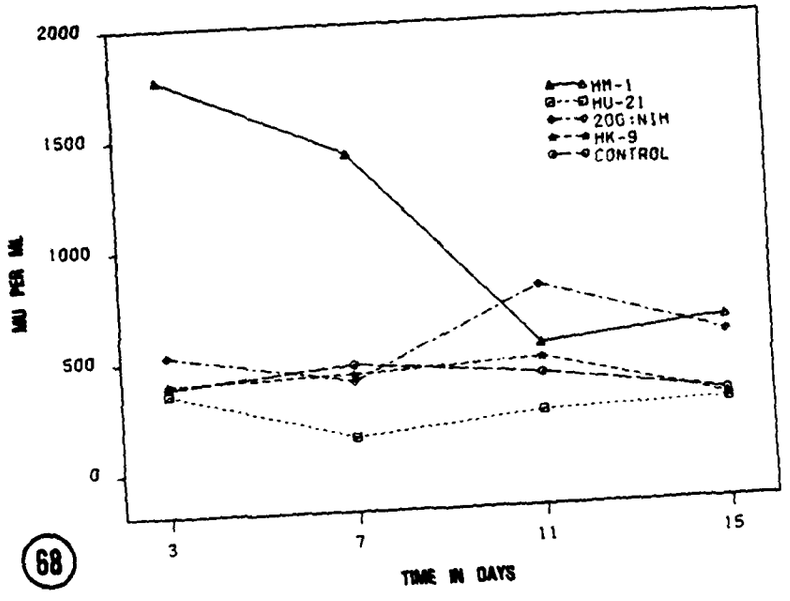

2000
1500
1000
500
0
MU PER ML
3
7
11
15
TIME IN DAYS
MM-1
MU-21
20G:NIM
MK-9
CONTROL
68

TABLE 30

ANALYSIS OF VARIANCE IN ASPARTATE AMINOTRANSFERASE AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	131451.42	3	43817.14	3.66332	.025
HM-1 vs other strains	78287.13	1	78287.13	6.54517	.016
HK-9 vs HU-21 + 200:NIH	2044.26	1	2044.26	.17091	.683
200:NIH vs HU-21	51120.03	1	51120.03	4.27387	.048
Time (T)	43155.80	3	14385.27	1.20268	.328
Linear	32180.25	1	32180.25	2.69042	.113
Quadratic	129.39	1	129.39	.01082	.918
Cubic	10846.15	1	10846.15	.90679	.349
Dose (D)	46287.17	3	15429.06	1.28994	.298
Control vs infected	29082.13	1	29082.13	2.43140	.131
Linear	850.78	1	850.78	.07113	.792
Quadratic	16354.26	1	16354.26	1.36729	.252
S x T interaction	216881.14	9	24097.90	2.01470	.077
S x D interaction	105357.27	9	11706.36	.97871	.479
T x D interaction	88664.89	9	9851.65	.82364	.600
Error	322948.42	27	11961.05		

TABLE 31

ANALYSIS OF VARIANCE IN ALANINE AMINOTRANSFERASE AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	77100.88	3	25700.29	3.81017	.021
HM-1 vs other strains	66352.08	1	62352.08	9.24395	.005
HK-9 vs HU-21 + 200:NIH	42.67	1	42.67	.00633	.937
200:NIH vs HU-21	14706.12	1	14706.12	2.18024	.151
Time (T)	51806.38	3	17268.79	2.56017	.076
Linear	32361.01	1	32361.01	4.79765	.037
Quadratic	132.25	1	132.25	.01961	.890
Cubic	19313.11	1	19313.11	2.86325	.102
Dose (D)	22822.50	3	7607.50	1.12784	.355
Control vs infected	21336.33	1	21336.33	3.16320	.087
Linear	338.00	1	338.00	.05011	.825
Quadratic	1148.17	1	1148.17	.17022	.683
S x T interaction	156601.75	9	17400.19	2.57965	.027
S x D interaction	74514.62	9	8279.40	1.22746	.320
T x D interaction	61436.12	9	6826.24	1.01202	.455
Error	182119.75	27	6745.18		

TABLE 32

ANALYSIS OF VARIANCE IN LACTATE DEHYDROGENASE AT FOUR LEVELS OF STRAIN, TIME, AND DOSE

WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	3204689.12	3	1068229.71	6.67099	.002
HM-1 vs other strains	2703701.33	1	2703701.33	16.88434	<.0005
HK-9 vs HU-21 + 200:NIH	237.51	1	237.51	.00148	.970
200:NIH vs HU-21	500700.28	1	500750.28	3.12713	.088
Time (T)	658477.00	3	219492.33	1.37071	.273
Linear	655944.20	1	655944.20	4.09630	.053
Quadratic	1764.00	1	1764.00	.01102	.917
Cubic	768.80	1	768.80	.00480	.945
Dose (D)	713884.38	3	237961.46	1.48605	.241
Control vs infected	420376.33	1	420376.33	2.62521	.117
Linear	2888.00	1	2888.00	.01804	.894
Quadratic	290620.04	1	290620.04	1.81489	.189
S x T interaction	2345621.38	9	260624.60	1.62757	.157
S x D interaction	1944367.00	9	216040.78	1.34915	.259
T x D interaction	1496495.12	9	166277.24	1.03838	.436
Error	4323529.00	27	160130.70		

TABLE 33

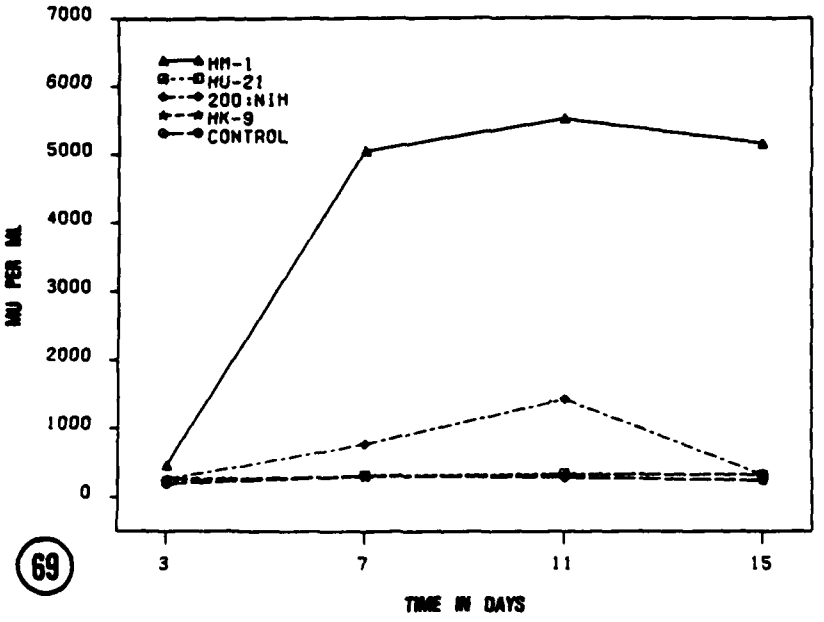
ANALYSIS OF VARIANCE IN ALKALINE PHOSPHATASE AT FOUR LEVELS OF STRAIN, TIME, AND DOSE

WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

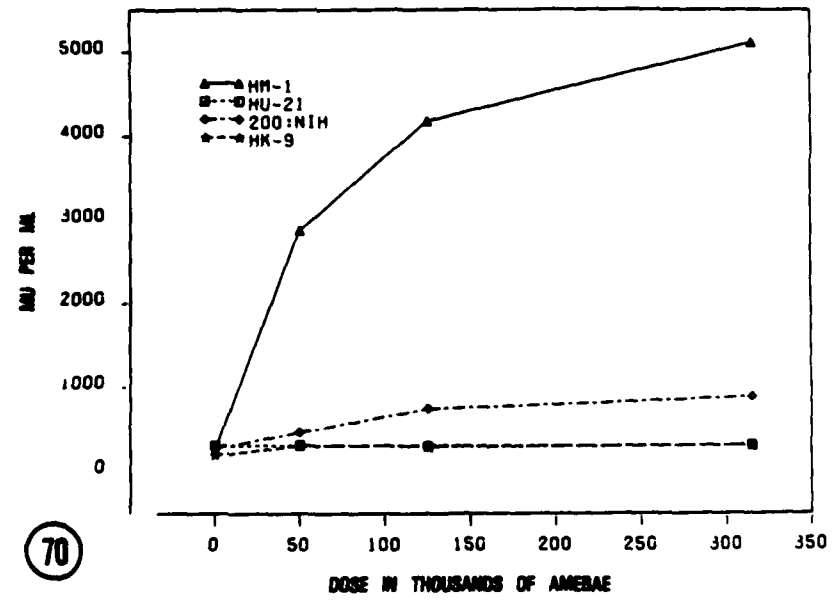
Source of Variation	SS	d.f.	MS	F	p
Strain (S)	88849692.12	3	29616564.04	21.80155	<.0005
HM-1 vs other strains	87885468.75	1	87885468.75	64.69484	<.0005
HK-9 vs HU-21 + 200:NIH	309855.38	1	309855.38	.22809	.637
200:NIH vs HU-21	654368.00	1	654368.00	.48170	.494
Time (T)	14028819.38	3	4676273.12	3.44233	.031
Linear	7233037.81	1	7233037.81	5.32443	.029
Quadratic	6721056.25	1	6721056.25	4.94755	.035
Cubic	74725.31	1	74725.31	.05501	.816
Dose (D)	17545464.88	3	5848488.29	4.30523	.0_3
Control vs infected	13979525.33	1	13979525.33	10.29070	.003
Linear	3929824.50	1	3929824.50	2.59840	.119
Quadratic	36115.04	1	36115.04	.02659	.872
S x T interaction	27180602.00	9	3020066.89	2.22315	.053
S x D interaction	36688702.50	9	4076522.50	3.00084	.013
T x D interaction	10262476.25	9	1140275.14	.83939	.587
Error	36678465.875	27	1358461.69907		

Figure 69. Alkaline phosphatase measured in serum samples during the course of experimental hepatic amebiasis in hamsters. Strain HM-1 caused extremely high amounts of ALP by seven days whereas 200:NIH caused a much less dramatic increase at 11 days.

Figure 70. Serum alkaline phosphatase related to inoculum size. Elevated ALP was dose dependent in the HM-1 group in which extremely high levels were reached. Strain 200:NIH at higher doses caused moderate increases in the enzyme.


7000
6000
5000
4000
3000
2000
1000
0
MU PER ML
HM-1
HU-21
200:NIH
HK-9
CONTROL
3
7
11
15
TIME IN DAYS

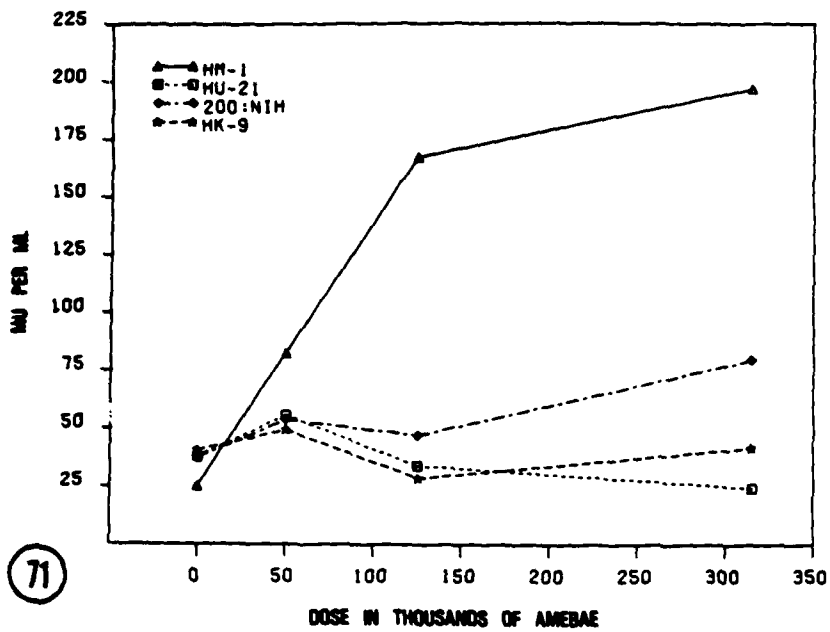
69


5000
4000
3000
2000
1000
0
MU PER ML
HM-1
HU-21
200:NIH
HK-9
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

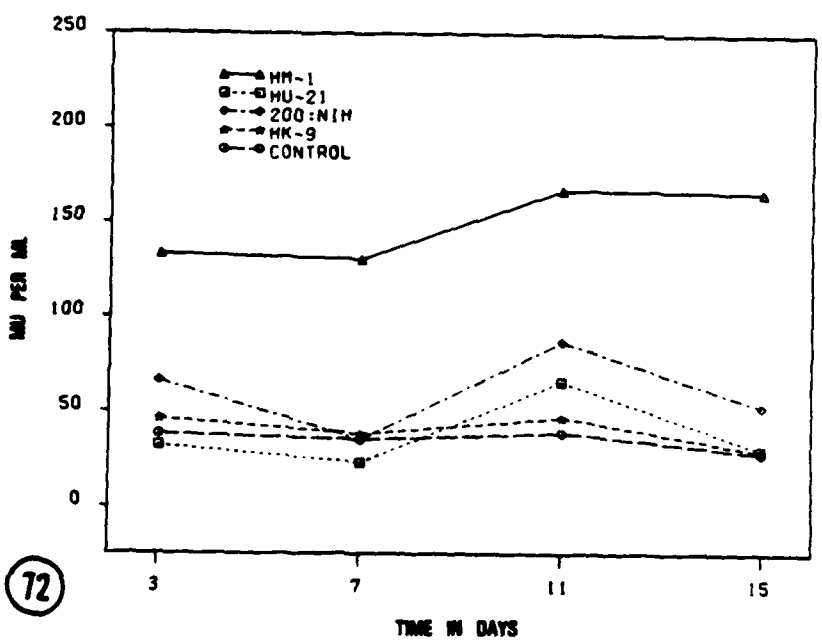
70

Figure 71. Relationship of inoculum size to aldolase in serum. The amount of ALD was positively related to increasing numbers of HM-1 amebae, but the highest dose of 200:NIH was required to elevate this enzyme. Compare figure 70.

Figure 72. Temporal changes in serum aldolase. Strain HM-1 caused significant elevation of ALD throughout the experiment. Notice the absence of interaction between strain and time.


225
200
175
150
125
100
75
50
25
MM-1
MU-21
200:NIH
MK-9
MU PER ML
0
50
100
150
200
250
300
350
DOSE IN THOUSANDS OF AMEBAE

71


250
200
150
100
50
0
MM-1
MU-21
200:NIH
MK-9
CONTROL
MU PER ML
3
7
11
15
TIME IN DAYS

72

TABLE 34

ANALYSIS OF VARIANCE IN ALDOLASE AT FOUR LEVELS OF STRAIN, TIME, AND DOSE WITH ORTHOGONAL CONTRASTS FOR MAIN EFFECTS

Source of Variation	SS	d.f.	MS	F	p
Strain (S)	68130.29687	3	22710.09896	9.41665	<.0005
HM-1 vs other strains	65527.13021	1	65527.13021	27.17056	<.0005
HK-9 vs HU-21 + 200:NIH	392.04167	1	392.04167	.16256	.690
200:NIH vs HU-21	2211.12500	1	2211.12500	.91683	.347
Time (T)	6362.79687	3	2120.93229	.87944	.464
Linear	371.95312	1	371.95312	.15423	.698
Quadratic	337.64062	1	337.64062	.14000	.711
Cubic	5653.20312	1	5653.20312	2.34408	.137
Dose (D)	21453.42187	3	7151.14062	2.96519	.050
Control vs infected	16078.38021	1	16078.38021	6.66683	.016
Linear	5227.53125	1	5227.53125	2.16757	.153
Quadratic	147.51042	1	147.51042	.06116	.807
S x T interaction	2228.64062	9	247.62674	.10268	.999
S x D interaction	59927.01562	9	6658.55729	2.76094	.020
T x D interaction	24518.01562	9	2724.22396	1.12959	.377
Error	65115.79687	27	2411.69618		

TABLE 35

PATHOLOGY AND PATHOPHYSIOLOGY OF HEPATIC AMEBIASIS CAUSED BY AXENIC HM-1 AMEBAE IN HAMSTERS

	Time after Infection							
	3 days		7 Days		11 Days		15 Days	
Variable	Infected*	Control**	Infected	Control	Infected	Control	Infected	Control
General pathology								
Lesion count	21.0	0.0	13.0	0.0	8.0	0.0	4.7	0.0
Largest lesion size (mm)								
Length	8.67	. . .	20.67	. . .	20.67	. . .	33.50	. . .
Width	5.67	. . .	16.67	. . .	18.00	. . .	22.00	. . .
Depth	5.50	. . .	9.33	. . .	8.67	. . .	11.00	. . .
Liver weight (g)	4.070	4.765	8.700	4.200	8.630	4.958	10.990	4.850
Spleen weight (mg)	131.80	123.00	480.47	84.80	359.87	80.50	403.27	69.25
Pathophysiology								
Change in body weight (g)	-5.53	-5.75	-1.03	+0.55	-3.17	-0.15	+3.93	+2.10
Hematology								
RBC count (millions/mm^3)	6.003	7.028	6.257	6.832	5.380	7.080	6.140	7.308
Hematocrit (%)	42.83	48.50	39.83	50.38	36.33	52.88	44.67	53.88
WBC count (thousands/mm^3)	7.993	6.077	11.237	3.715	7.324	4.716	4.987	4.123
Neutrophils (%)	64.3	40.0	55.0	42.8	49.0	28.2	39.0	42.8
Lymphocytes (%)	29.0	54.8	35.7	52.8	42.7	68.0	55.3	55.0
Monocytes (%)	6.0	3.8	4.0	2.8	3.3	3.2	2.7	1.0
Eosinophils (%)	0.3	1.5	5.0	1.8	4.3	0.5	3.0	1.2
Basophils (%)	0.3	0.0	0.3	0.0	0.7	0.0	0.0	0.0

TABLE 35—Continued

Variable	Time after Infection: 3 days		7 Days		11 Days		15 Days	
	Infected*	Control**	Infected	Control	Infected	Control	Infected	Control
Serum protein electrophoresis								
Total protein (g/100 ml)	5.27	6.12	5.20	6.00	5.67	6.28	7.43	5.55
A:G	0.998	1.553	0.771	1.666	0.789	1.67	0.704	1.68
Albumin (%)	49.70	60.20	43.53	62.32	44.07	62.15	37.53	62.48
Alpha-1 (%)	11.7	10.92	10.93	10.72	10.57	11.42	9.10	9.32
Alpha-2 (%)	12.20	11.20	11.17	7.92	9.03	8.42	7.77	8.30
Beta (%)	23.40	14.02	23.27	14.60	22.20	13.60	16.63	15.50
Gamma (%)	3.67	3.62	11.10	4.42	14.17	4.50	29.00	4.40
Complement fixation (GMT***)	25.4	1.0	161.3	13.5	161.3	9.5	256.0	3.4
Liver function tests								
Total bilirubin (mg/100 ml)	0.20	0.48	0.37	0.28	0.30	0.45	0.47	0.50
Aspartate aminotransferase mU/ml)	441.0	62.5	252.3	82.8	84.3	104.0	74.3	49.8
Alanine aminotransferase (mU/ml)	371.3	42.2	199.7	42.0	64.3	67.5	32.7	36.8
Lactate dehydrogenase (mU/ml)	1769.0	383.0	1413.3	464.0	531.0	396.0	626.3	289.0
Aldolase (mU/ml)	132.7	37.5	130.0	35.2	167.0	39.0	166.0	28.5
Alkaline phosphatase (mU/ml)	464.0	192.5	5046.7	316.8	5514.0	281.5	5159.3	240.8

* Mean values for three infected animals at each time interval.

** Mean values for four sham-operated control animals at each time interval.

*** GMT = geometric mean titer; a value of 1.0 represents a reciprocal CF titer <4.

TABLE 36

PATHOLOGY AND PATHOPHYSIOLOGY OF HEPATIC AMEBIASIS CAUSED BY AXENIC 200:NIH AMEBAE IN HAMSTERS

	Time after Infection							
	3 days		7 Days		11 Days		15 Days	
Variable	Infected*	Control**	Infected	Control	Infected	Control	Infected	Control
General pathology								
Lesion count	17.3	0.0	1.0	0.0	21.7	0.0	0.3	0.0
Largest lesion size (mm)								
Length	4.03	. . .	14.50	. . .	19.00	. . .	6.00	. . .
Width	2.37	. . .	13.00	. . .	13.00	. . .	3.00	. . .
Depth	2.37	. . .	6.50	. . .	9.67	. . .	3.00	. . .
Liver weight (g)	3.593	4.765	4.810	4.200	9.273	4.958	4.393	4.850
Spleen weight (mg)	97.33	123.00	172.53	84.80	356.27	80.50	67.93	69.25
Pathophysiology								
Change in body weight (g)	-6.40	-5.75	-4.80	+0.55	-7.70	-0.15	+1.57	+2.10
Hematology								
RBC count (millions/mm^3)	6.350	7.028	6.617	6.832	6.477	7.080	7.510	7.308
Hematocrit (%)	43.17	48.50	46.83	50.38	41.17	52.88	51.33	53.88
WBC count (thousands/mm^3)	6.398	6.077	13.378	3.715	13.683	4.716	5.120	4.123
Neutrophils (%)	49.0	40.0	45.3	42.8	60.7	28.2	39.3	42.8
Lymphocytes (%)	43.3	54.8	40.3	52.8	31.3	68.0	55.7	55.0
Monocytes (%)	7.3	3.8	5.3	2.8	5.0	3.2	1.7	1.0
Eosinophils (%)	0.3	1.5	9.0	1.8	3.0	0.5	3.3	1.2
Basophils (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 36—*Continued*

Variable	Time after Infection: 3 days		7 Days		11 Days		15 Days	
	Infected*	Control**	Infected	Control	Infected	Control	Infected	Control
Serum protein electrophoresis								
Total protein (g/100 ml)	5.03	6.12	6.30	6.00	5.57	6.28	5.43	5.55
A:G	1.142	1.553	1.117	1.666	0.712	1.671	1.842	1.679
Albumin (%)	53.00	60.20	50.83	62.32	41.53	62.15	64.70	62.48
Alpha-1 (%)	10.80	10.92	12.40	10.72	10.50	11.42	8.57	9.32
Alpha-2 (%)	12.23	11.20	10.30	7.92	9.60	8.42	8.00	8.30
Beta (%)	20.17	14.02	20.50	14.60	21.73	13.60	13.93	15.50
Gamma (%)	3.77	3.62	5.97	4.42	16.67	4.50	4.77	4.40
Complement fixation (GMT***)	10.1	1.00	40.3	13.5	322.5	9.5	2.5	3.4
Liver function tests								
Total bilirubin (mg/100 ml)	0.23	0.48	0,40	0.28	0.43	0.45	0.47	0.50
Aspartate aminotransferase mU/ml)	126.3	62.5	79.0	82.8	265.3	104.0	151.7	49.8
Alanine aminotransferase (mU/ml)	100.3	42.2	31.3	42.0	191.3	67.5	56.3	36.8
Lactate dehydrogenase (mU/ml)	524.0	383.0	389.3	464.0	790.7	396.0	557.0	289.0
Aldolase (mU/ml)	65.7	37.5	35.0	35.2	87.0	39.0	52.7	28.5
Alkaline phosphatase (mU/ml)	259.7	192.5	772.3	316.8	1424.7	281.5	320.7	240.8

* Mean values for three infected animals at each time interval.

** Mean values for four sham-operated control animals at each time interval.

*** GMT = geometric mean titer; a value of 1.0 represents a reciprocal CF titer <4.

Effects of Immunosuppression in the Hamster Model

Axenically cultivated trophozoites of Entamoeba histolytica caused granulomatous inflammation in the liver of experimentally infected hamsters. Unanue (1978) pointed out the association of cell-mediated immunity with granulomatous inflammation in a variety of infectious diseases. Then this type of cellular response to E. histolytica could also be mediated by T lymphocytes. Because immunosuppression can exacerbate amebiasis in patients, immunosuppression of infected hamsters should also alter the outcome of experimental amebiasis. The hypothesis about CMI in experimental hepatic amebiasis of hamsters could be tested by immunosuppression of the host.

Partial ablation of the immune system was used to study the role of cell-mediated immunity in hepatic amebiasis of hamsters. Fifty thousand HM-1 trophozoites were given by the intraportal route to three normal hamsters, three hamsters injected with normal rabbit serum, and 15 immunosuppressed hamsters. Methods of immunosuppression included cyclophosphamide, antilymphocyte serum, antithymocyte serum, and a combination of ALS or ATS with neonatal thymectomy. Necropsies were performed six days after infection. Tables 61-65 in appendix 4 include the experimental data.

Use of Cyclophosphamide

The dose response to cyclophosphamide was determined in a preliminary experiment with LHC/Lak hamsters, which received a single intraperitoneal injection of the drug (table 37). A dose of 22.5 mg cyclophosphamide per 100 g body weight was selected because only one of five animals given this dose died within 14 days, although the leukocyte count was very low in those animals five days after receiving the drug. Doses of 10-12.5 mg CY per 100 g body weight have been used routinely for immunosuppression of hamsters (Kurata et al. 1981, Ohtaki 1981).

Lymphocyte Blast Transformation

The efficacy of immunosuppression was assessed by mitogen stimulation of spleen cells harvested from uninfected hamsters 24 hours after ending the immunosuppressive treatment. Lymphocyte blast transformation by the method of Monjan and Mandell (1980) was done in triplicate on spleen cells from two uninfected animals in each group. Two mitogenic lectins, enterotoxin lipopolysaccharide (LPS) to stimulate B cells and concanavalin A (ConA) to stimulate T cells (Hart and Stein-Streilein 1981), were used to measure the blastogenic response of lymphocytes from the spleen. All of the animals had an intact thymus. Control

animals were either untreated or were injected with saline or normal rabbit serum.

The data in table 38 show uptake of ^{3}H-thymidine by spleen cells stimulated with LPS or ConA following immunosuppression of donor animals. Each of the three immunosuppressed groups (CY, ALS, ATS) should be compared with the control animals (untreated, saline, and NRS). For example, when compared with LPS values for control animals, the specific uptake of ^{3}H-thymidine (or stimulation index) following an LPS pulse indicated a depleted population of B cells in animals given cyclophosphamide. Likewise, ConA values indicated depressed numbers of T cells in the CY-treated animals. In animals treated with ALS the B cell response was normal, but the T cell population was ablated. ATS reduced the number of B cells in the spleen but was not as effective against T cells, although ConA values for the ATS group were considerably lower than controls. These data indicated that animals infected with amebae following these regimens for immunosuppressive agents should have had a significant degree of immunosuppression at the time of infection. Animals that received ALS or ATS prior to amebic infection were given five additional injections of the

antiserum. Two groups of infected animals had been neonatally thymectomized in addition to receiving ALS or ATS, but blast transformation was not done.

General Pathology

This experiment must be regarded as preliminary because only a few animals were available. Nevertheless, selected data are presented.

Table 39 summarizes information gathered about the number and size of amebic liver lesions in the immunosuppressed hamsters. The appropriate control for animals treated with cyclophosphamide was the group designated HM-1 control, which received only amebae. The appropriate control for the other groups was the NRS control group, which received NRS in addition to amebae. Animals treated with CY had fewer lesions and smaller ones than HM-1 controls, and the difference in the number of lesions was significant at the .05 level by the Duncan multiple range test. Although the number of lesions in the NRS controls was fewer than in the other immunosuppressed animals, the difference was not statistically significant. For all three dimensions of the lesions, immunosuppressed animals had significantly smaller lesions than the two control groups ($p = .01$).

When liver weights (table 40) were compared among the treatment groups, the difference was not significant. None of the livers was greatly enlarged, probably because necropsy was done six days after infection. In an earlier experiment, animals infected with HM-1 amebae developed hepatomegaly seven days or more after infection. Thus, one would need to follow immunosuppressed animals longer than six days in order to see a difference between them and controls.

Overall variation in spleen weight (table 40) was not significantly different among treatment groups, but one of the orthogonal contrasts turned up an interesting finding. Animals treated with CY prior to amebic infection had significantly smaller spleens than other immunosuppressed animals ($p = .007$).

Pathophysiology

The blood picture was also expected to be different among treatment groups. The data in table 41 show some differences among treatment groups with respect to the number of leukocytes in the peripheral blood. The differences were not statistically significant, however. Overall variation among treatment groups was highly significant with respect to the percentage of neutrophils ($p = .0002$), but this difference disappeared when the actual number of neutrophils in the peripheral blood was

calculated. The higher proportion of neutrophils in the blood resulted from the paucity of lymphocytes in the blood of animals that received antiserum or normal rabbit serum. Not only the proportion of lymphocytes but also the actual number of lymphocytes was much smaller in the animals that received rabbit serum ($p < .00005$). The Duncan multiple range test did not detect a significant difference between NRS controls and the other groups that received antiserum, but all of them had significantly fewer lymphocytes both in proportion and actual number than HM-1 controls and CY-treated animals ($p \leq .01$). The nonspecific suppression of lymphocytes in the peripheral blood would not have been detected without including control animals that received normal rabbit serum.

Another interesting result from injecting rabbit serum into hamsters was the presence of a pre-albumin peak on electrophoretic patterns of serum proteins (figure 73). Only hamsters that received antiserum or normal serum from rabbits had pre-albumin.

Measuring gamma globulin was not enlightening because infected HM-1 control animals that had not been immunosuppressed had one of the lowest levels (table 42). Variation among groups was not significant at the .01 level,

but the Duncan procedure found the CY group and HM-1 control group to be significantly different ($p \leq .01$) from the ATS group, animals that had the highest amounts of gamma globulin.

Antibodies to Entamoeba histolytica measured by complement fixation (table 42) varied significantly among the groups (table 43). Orthogonal contrasts (table 44) indicated that the two control groups differed significantly from the immunosuppressed animals, and the CY group had much less antibody than the other immunosuppressed animals. In fact, the CY-treated animals did not have detectable amounts of antibody (table 42). By the Duncan test the CY group differed significantly with respect to CF titer from all the other groups except thymectomized animals that received ATS ($p \leq .01$).

Histopathology

Livers from the animals in this experiment were examined pathologically. Liver lesions from the two control groups (figure 74) were similar to those described earlier for animals infected with HM-1 five days previously. Animals treated with ATS had liver lesions (figures 75-77) similar to those of controls. The ALS group had less extensive necrosis of the liver (figure 78), and amebic lesions were less likely to coalesce into large

multiloculated ones. There was no macroscopic nor microscopic evidence of a thymus in animals that had been neonatally thymectomized. The TX + ALS group had no large necrotic lesions although small yellowish ones were scattered throughout the liver. The most remarkable pathologic changes were seen in thymectomized animals that received ATS (figure 79). There were no large accumulations of mononuclear cells in any of the lesions. In other respects the cellular reaction to amebic invasion was less intense but similar to that of intact hamsters. At the leading edge of growing lesions, amebae encountered an acute inflammatory reaction of PMN and eosinophils (figure 80). In their wake, amebae left dead inflammatory cells and degenerating liver tissue while polymorphonuclear leukocytes infiltrated the fibrotic layer (figure 81). Gross pathology of these livers was similar to that of infected control animals, but inflammation of the portal tracts was not observed. The CY group did not have large necrotic lesions, but focal necrosis with granulocytic infiltration was present (figure 82). There were very few mononuclear cells. Inflammation of the portal tracts (figure 83) was moderate, and acute vasculitis was seen occasionally.

Further Experimentation with Cyclophosphamide

Cyclophosphamide seemed to have the opposite effect of mitigating hepatic amebiasis in the hamster compared to the other means of immunosuppression, which exacerbated the infection. Therefore, another experiment was done using a larger number of animals to confirm this unexpected result. One group of ten hamsters was infected with 315 000 HM-1 trophozoites 24 hours after the hamsters had been given 22.5 mg of cyclophosphamide per 100 g body weight. A control group of ten hamsters was infected with the same number of amebae but received no CY. Five animals from both groups were necropsied at seven days, and the remaining animals were necropsied 15 days after infection.

Table 45 summarizes mortality that occurred in this experiment. Some deaths were expected with a CY dose of 22.5 mg/100 g (table 37), but CY administered prior to infection with amebae did not cause excess mortality in this experiment.

The number of liver lesions summarized in table 46 was similar for the CY group and controls at seven days. Immunosuppressed hamsters had fewer lesions 15 days after infection, however. Lesions in control animals were much larger than lesions in the CY group (table 47). Gross pathology revealed minimal damage to the livers of

CY-treated animals compared to massive necrosis in control animals.

Histopathology was able to clarify some of these findings. In this experiment infected control animals had the same pathological changes in the liver at seven days as those described from animals infected with HM-1 in an earlier experiment. In contrast, the CY group had small circumscribed granulomas seven days after infection (figure 84). Amebae were very sparse and did not invade adjacent liver tissue (figure 85). In areas of the liver apart from the amebic lesions there was some multifocal hepatocellular degeneration with acute inflammation. Portal tracts had pronounced inflammation (figure 86) consisting of mostly PMN with mononuclear cells and eosinophils also present. No proliferation of bile ducts was seen. Amebic lesions in CY-treated hamsters were not only smaller but also less advanced than comparable lesions in control animals.

Fifteen days after infection the livers of infected control animals exhibited typical pathological changes described in an earlier experiment. In marked contrast, the CY group had few lesions; and those were actually small healing granulomas (figures 87-88). Numerous macrophages were at the center of lesions, but some central necrosis

was still present. A thin wall of fibroblasts encircled the macrophages and compartmentalized the lesions. Chronic inflammation including plasma cells and numerous eosinophils surrounded the fibrosis. Very few amebae were seen. Thus, the absence of amebae in older lesions and their paucity in seven-day lesions from CY-treated hamsters suggested that CY had a deleterious effect on the reproduction of amebae in hamster liver.

Summary of Immunosuppression in the Hamster Model

The efficacy of immunosuppression was evaluated by lymphocyte blast transformation of spleen cells from hamsters that were immunosuppressed by the same methods (with exception of neonatal thymectomy) used for other hamsters up to the time of their infection with HM-1 amebae. Results of blast transformation indicated that ALS by itself depleted T cells while ATS depleted B cells and to a lesser extent T cells from the spleen. Cyclophosphamide depleted both T and B lymphocytes. Neonatal thymectomy presumably helped deplete the T cell population (Lewis et al. 1981).

All of the means used for immunosuppression except cyclophosphamide resulted in more liver lesions compared to appropriate controls; therefore, immunosuppression seemed to exacerbate amebic infection, as expected. Antithymocyte serum following neonatal thymectomy or ATS alone were more

effective in this regard than the other immunosuppressive regimens. Although the number of lesions was greater in the ATS and TX + ATS groups, the lesions were smaller than NRS control lesions. Nevertheless, the total amount of amebic necrosis in the ATS or TX + ATS animals was greater. More severe disease resulted from immunosuppression by ATS alone or thymectomy with ATS. Antithymocyte serum alone or in combination with thymectomy caused a marked reduction of lymphocytes in the peripheral blood and low levels of antibody. In the TX + ATS group, mononuclear cells were notably absent from the cellular response to amebae in tissue. In other respects, the histopathology of amebic liver lesions from immunosuppressed hamsters, excluding CY-treated animals, was similar to but less intense than infected controls.

Another experiment with CY followed infected hamsters for 15 days when it was apparent that CY-treated animals had fewer lesions and much smaller ones than controls. Although 315 000 pathogenic HM-1 amebae had been given, they failed to thrive in the CY group. Cyclophosphamide apparently had a deleterious effect on the amebae, and the granulomatous cellular response resulted in partial healing of the lesions.

TABLE 37

DOSE RESPONSE OF HAMSTERS TO CYCLOPHOSPHAMIDE

Dose (mg/100 g BW)	Deaths/No. Animals in 14 Days	Leukocyte Count at 5 Days
0	0/5	6690
7.5	0/5	1820
15.0	0/5	1700
22.5	1/5	410
30.0	2/5	380
37.5	5/5	ND

TABLE 38

MITOGEN STIMULATION OF HAMSTER SPLEEN CELLS WITH LIPOPOLYSACCHARIDE AND CONCANAVALIN A

	Lipopolysaccharide (12.5 µg/ml)*				Concanavalin A (4µg/ml)*		
Treatment**	Background CPM	CPM***	Specific Uptake#	Stimulation Index##	CPM	Specific Uptake	Stimulation Index
Untreated	759.0	2286.7	1527.7	3.01	117281.2	116532.2	154.53
Saline###	679.2	1555.6	876.4	2.29	186364.4	185685.2	274.39
NRS###	753.5	2298.2	1544.7	3.05	255530.4	254776.9	339.12
CY@	861.6	992.0	130.4	1.15	14359.4	13497.8	16.67
ALS###	1119.2	3626.8	2507.6	3.24	14586.5	13467.3	13.03
ATS###	1036.8	608.5	0	.59	25568.6	24531.8	24.66

* Optimal dose of mitogen.

** NRS = normal rabbit serum; CY = cyclophosphamide; ALS = antilymphocyte serum; ATS = antithymocyte serum.

*** CPM = counts per minute representing the mean for two spleens with cultures done in triplicate for both mitogens.

\# Specific uptake = stimulated CPM minus background CPM.

Stimulation index = stimulated CPM ÷ background CPM.

Dosage was 1.25 cc/100 g body weight daily for two days before harvest of spleen cells.

@ Dosage was 22.5 mg/100 g body weight given i.p. one day before harvest of spleen cells.

TABLE 39

NUMBER AND SIZE OF LIVER LESIONS IN IMMUNOSUPPRESSED HAMSTERS INFECTED WITH 50 THOUSAND HM-1 TROPHOZOITES VIA THE PORTAL VEIN

Treatment Group*	No. Animals	Mean No. Lesions	Mean Size of Largest Lesion in mm		
			Length	Width	Depth
Cyclophosphamide					
HM-1 control	3	38.3	11.33	8.33	6.33
CY	3	1.0	2.50	1.00	1.00
Antiserum and Neonatal Thymectomy					
NRS control	3	2.3	10.33	7.67	6.67
ALS	3	8.3	4.33	3.00	3.00
ATS	4	18.0	5.38	3.70	3.20
TX + ALS	2	10.5	1.25	1.25	1.25
TX + ATS	3	23.3	4.83	3.50	3.17

* CY = cyclophosphamide; NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 40

LIVER AND SPLEEN WEIGHTS IN IMMUNOSUPPRESSED HAMSTERS INFECTED WITH 50 THOUSAND HM-1 TROPHOZOITES VIA THE PORTAL VEIN

Treatment Group*	No. Animals	Mean Liver Weight (g)	Mean Spleen Weight (mg)
HM-1 control	3	4.943	206.87
CY	3	4.773	132.00
NRS control	3	4.767	257.73
ALS	3	4.797	355.60
ATS	4	4.675	319.50
TX + ALS	2	4.060	260.00
TX + ATS	3	4.110	227.87

* CY = cyclophosphamide; NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 41

LEUKOCYTE COUNT, NEUTROPHILS, AND LYMPHOCYTES IN IMMUNOSUPPRESSED HAMSTERS INFECTED WITH 50 THOUSAND HM-1 TROPHOZOITES VIA THE PORTAL VEIN

Treatment Group*	No. Animals	Leukocytes (per cu mm)	Neutrophils %	Neutrophils No. per cu mm	Lymphocytes %	Lymphocytes No. per cu mm
HM-1 control	3	8236.3	55.0	4514.8	39.7	3267.9
CY	3	11964.0	63.7	7641.1	31.3	3719.4
NRS control	3	9584.3	74.3	7194.0	17.7	1764.4
ALS	3	10137.0	80.0	8122.2	10.7	1064.9
ATS	4	7702.5	83.0	7301.3	9.7	685.0
TX + ALS	2	6964.5	87.5	6026.1	11.5	841.6
TX + ATS	3	5685.3	91.0	5167.8	5.0	287.2

* CY = cyclophosphamide; NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

Figure 73. Serum protein electrophoresis. Hamster injected with antiserum and infected with HM-1 trophozoites.

(73)

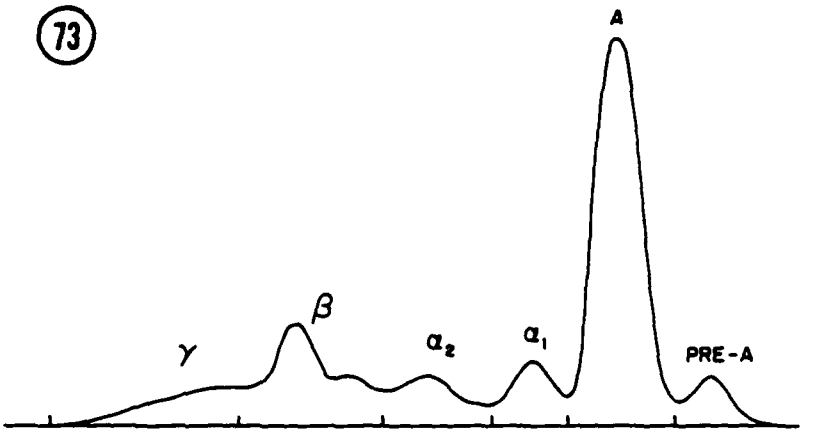


TABLE 42

GAMMA GLOBULIN AND COMPLEMENT FIXATION TITERS IN IMMUNOSUPPRESSED HAMSTERS INFECTED WITH 50 THOUSAND HM-1 TROPHOZOITES VIA THE PORTAL VEIN

Treatment Group*	No. Animals	Gamma Globulin (% total protein)	CF GMT**
HM-1 control	3	5.00	25.40
CY	3	4.73	1.00***
NRS control	3	7.70	20.16
ALS	3	8.07	16.00
ATS	4	9.82	11.31
TX + ALS	2	6.20	16.00
TX + ATS	3	5.40	4.00

* CY = cyclophosphamide; NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

** GMT = geometric mean titer.

*** Reciprocal titer <4.

TABLE 43

ANALYSIS OF VARIANCE IN COMPLEMENT FIXATION TITER*

AMONG IMMUNOSUPPRESSED HAMSTERS INFECTED WITH 50 THOUSAND

HM-1 TROPHOZOITES VIA THE PORTAL VEIN

Source of Variation	SS	d.f.	MS	F	p
Between groups	4.4317	6	.7386	7.961	.0007
Within groups	1.2989	14	.0928		

* Log_{10} transformation.

TABLE 44

ORTHOGONAL CONTRASTS FOR COMPARISON OF TREATMENT MEANS

FOR COMPLEMENT FIXATION TITER

Contrast	Difference between means	SE	t*	p**
HM-1 & NRS controls vs immunosuppressed	5.4185	1.4818	3.657	.003
HM-1 control vs NRS control	-.1003	.2487	-.403	.693
CY vs other immuno-suppressed	-4.0639	.7914	-5.135	<.0005
ALS vs ATS	.7526	.3625	2.076	.057
ALS + ATS vs TX	.4515	.3625	1.246	.233
ALS + ATS vs TX interaction	-.4515	.3625	-1.246	.233

* 14 d.f.

** Two-tailed.

Figure 74. Hepatic amebiasis caused by HM-1 trophozoites in a hamster that received normal rabbit serum. Chronic inflammation in response to caseous necrosis was similar to that seen in hamsters receiving only amebae. Compare figure 11. 6 days, H&E, X100.

Figure 75. Thrombophlebitis secondary to hepatic amebiasis in a hamster that received antithymocyte serum. The pathological changes were similar to those in figures 11 and 20 representing histopathology in hamsters that received only amebae. 6 days, PAS, X100.


75

Figure 76. Hematophagous trophozoites (A) invading liver tissue by extension from adjacent necrotic foci in a hamster immunosuppressed with ATS. Chronic inflammation was less intense near the amebae than in their wake. Compare figure 11. 6 days, H&E, X250.

Figure 77. Fibrosis and granulomatous reaction to masses of HM-1 amebae in a hamster that received ATS. Compare figure 15. 6 days, H&E, X250.

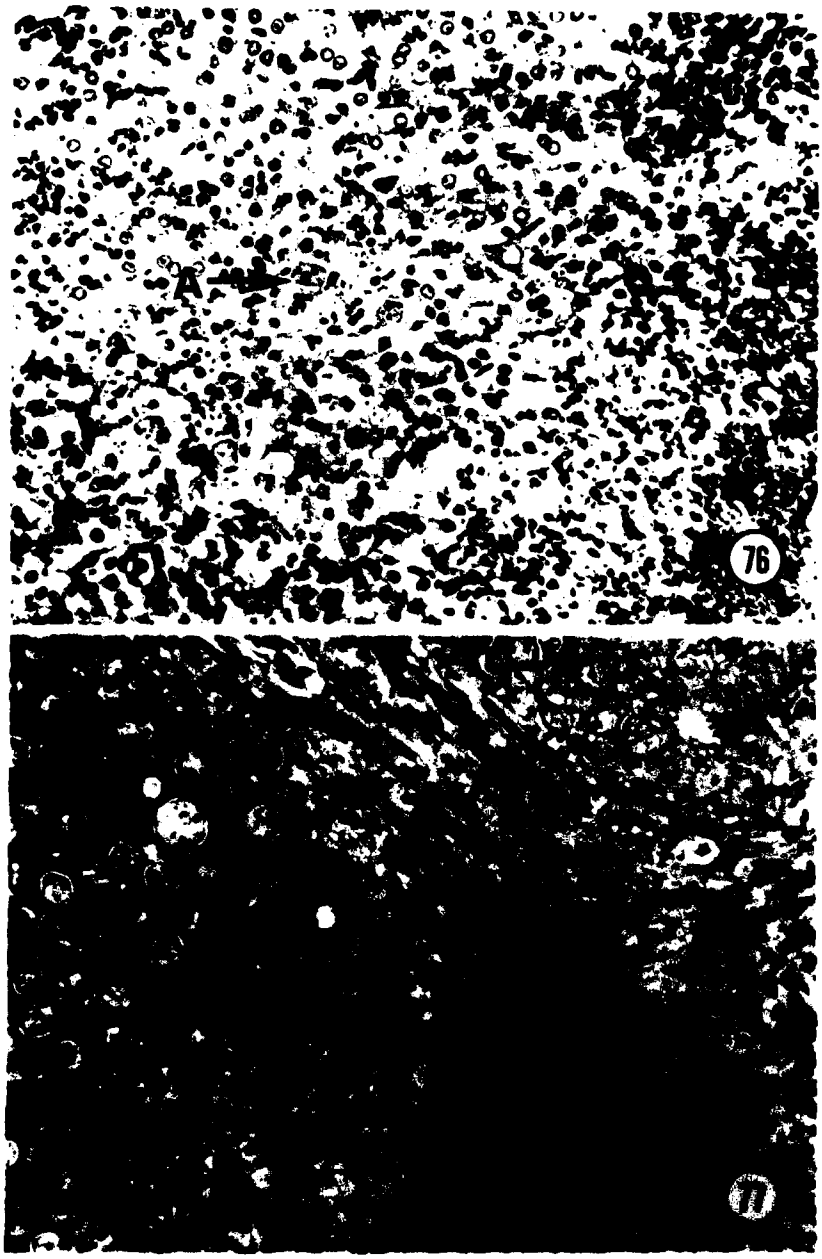

A
76
77

Figure 78. The most severe pathological changes seen in hamsters that received antilymphocyte serum. Hepatic necrosis was usually less extensive in the ALS group than in controls. 6 days, H&E, X100.

Figure 79. Hepatic amebiasis in a neonatally thymectomized hamster that received ATS. Mononuclear cells were nearly absent from the cellular reaction although granulocytes infiltrated the fibrotic response. Compare figures 14 and 17. 6 days, H&E, X100.

(a)
(b)

Figure 80. Advanding edge of an amebic lesion in the liver of a hamster neonatally thymectomized and given ATS. 6 days, H&E, X250.

Figure 81. Microabscess enclosed by fibrosis at the edge of an amebic liver lesion in a neonatally thymectomized hamster that received ATS. Note the almost complete absence of infiltrating mononuclear cells. 6 days, H&E, X250.

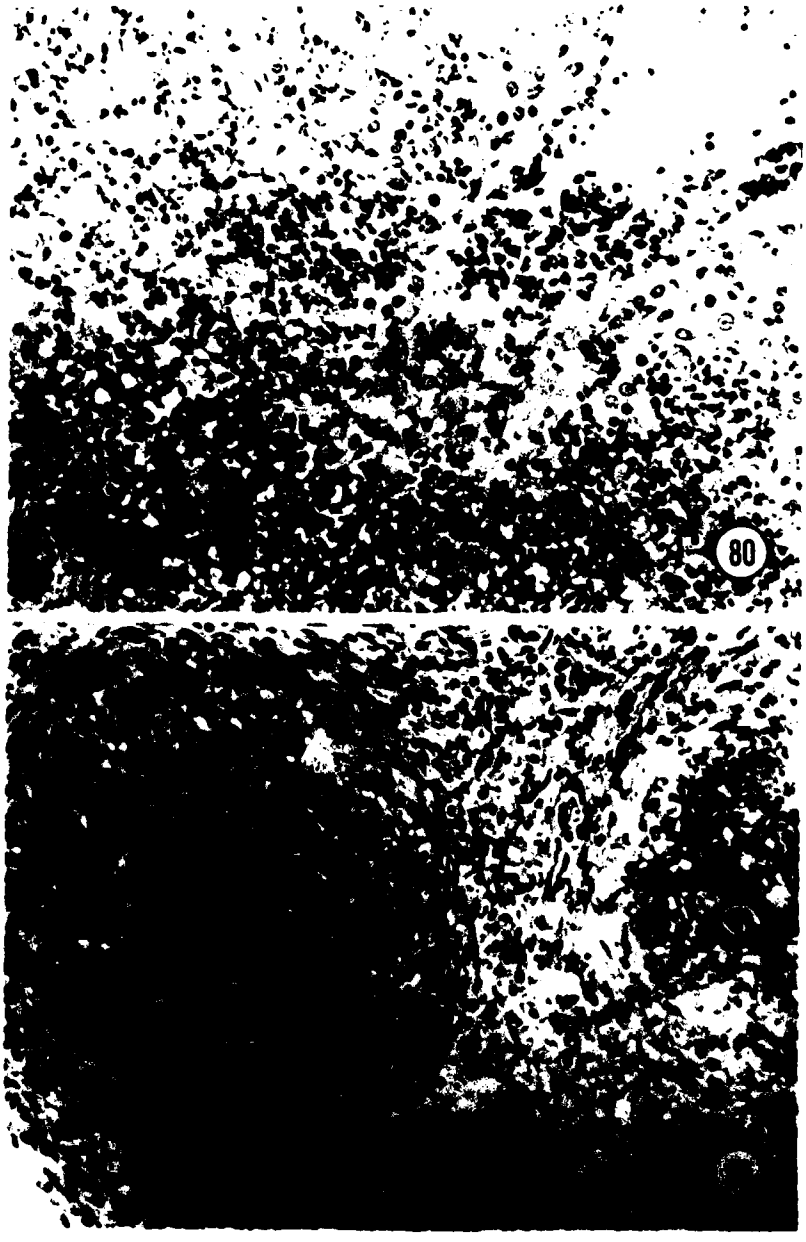

80

Figure 82. Focal necrosis with granulocytic infiltration in a hamster immunosuppressed with cyclophosphamide 24 h prior to intraportal inoculation with HM-1 amebae. 6 days, H&E, X400.

Figure 83. Periportal inflammation in a CY-treated hamster. 6 days, H&E, X100.

AD-A128 689 AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY TO ENTAMOEBA HISTOLYTICA(U) JOHNS HOPKINS UNIV BALTIMORE MD R G TAYLOR 15 APR 83 DAMD17-75-C-5001 4/6

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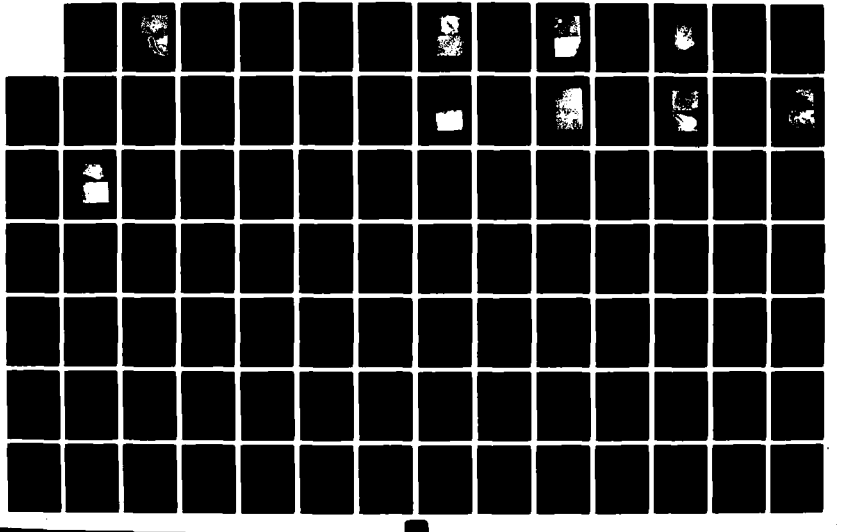

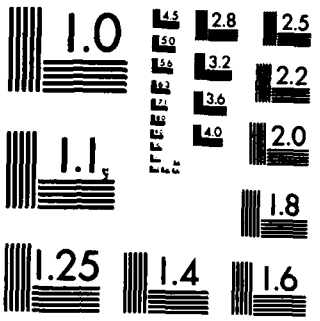

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

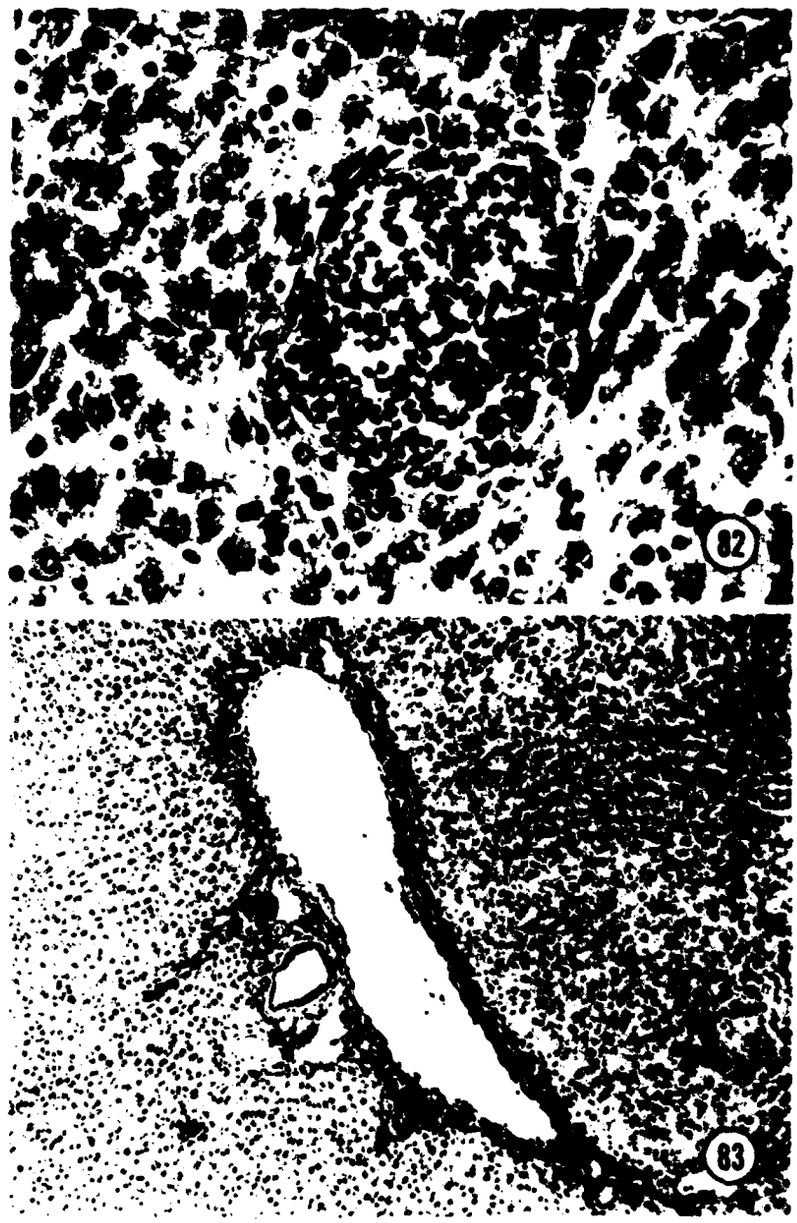
82
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TABLE 45

MORTALITY IN HAMSTERS INFECTED WITH 315 THOUSAND HM-1 TROPHOZOITES FOLLOWING IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE COMPARED TO INFECTED CONTROL ANIMALS

Time to Necropsy	CY Group	Control Group
7 days	0/5*	0/5
15 days	2/5**	1/5***

* Number of deaths prior to scheduled necropsy over number of animals in the group.

** Deaths occurred at 6 and 7 days.

*** Death occurred at 13 days.

TABLE 46

NUMBER OF LIVER LESIONS IN HAMSTERS INFECTED WITH 315 THOUSAND TROPHOZOITES FOLLOWING IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE COMPARED TO INFECTED CONTROL ANIMALS

Time to Necropsy		CY Group	Control Group
7 days		2	3
		2	4
		10	13
		4	4
		1	0
	Mean	3.8	4.8
15 days		0	6
		3	4
		0	4
		0	4
		1	2
	Mean	0.8	4.0

TABLE 47

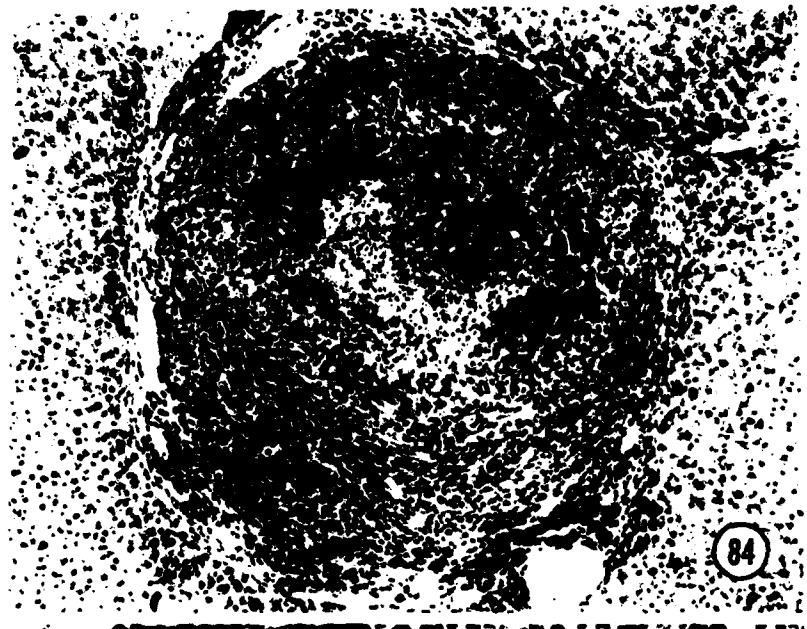
SIZE OF LARGEST LIVER LESIONS IN INFECTED HAMSTERS INFECTED WITH 315 THOUSAND HM-1 TROPHOZOITES FOLLOWING IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE COMPARED TO INFECTED CONTROL ANIMALS

	CY Group			Control Group		
Time to Necropsy	Length	Width	Depth	Length	Width	Depth
7 days	1.0*	1.0	1.0	31.0	26.0	9.0
	0.5	0.5	0.5	15.0	14.0	6.0
	1.0	1.0	1.0	22.0	15.0	6.0
	1.0	1.0	1.0	23.0	18.0	7.0
	1.0	1.0	1.0			
Mean	0.9	0.9	0.9	22.75	18.25	7.00
15 days	1.0	1.0	1.0	33.0	21.0	13.0
	2.0	1.0	1.0	21.0	17.0	12.0
				42.0	37.0	13.0
				40.0	36.0	19.0
				43.0	32.0	18.0
Mean	1.5	1.0	1.0	35.80	28.60	15.00

* Measurements in mm.

Figure 84. Small amebic granuloma in a hamster that was immunosuppressed with cyclophosphamide 24 h prior to infection. 7 days, H&E, X100.

Figure 85. Enlargement of figure 84. Chronic inflammation in response to antigenic stimulation by amebae (arrow). 7 days, H&E, X400.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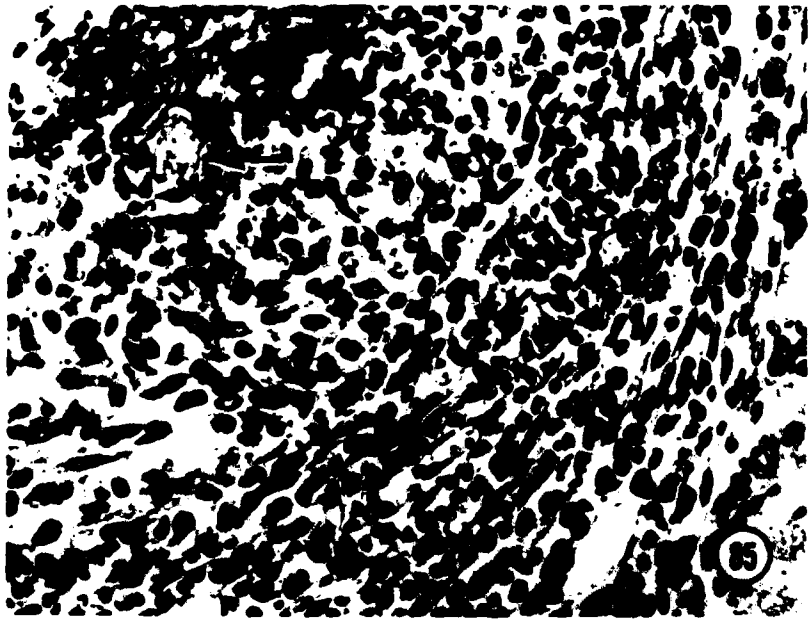

85

Figure 86. Inflammation surrounding a portal vein (PV) and megakaryocytes (MK) in the hepatic parenchyma of a CY-treated hamster. 7 days, H&E, X250.

Figure 87. Granulomatous reaction in the liver of a CY-treated hamster 15 days after intraportal inoculation with amebae. H&E, X100.

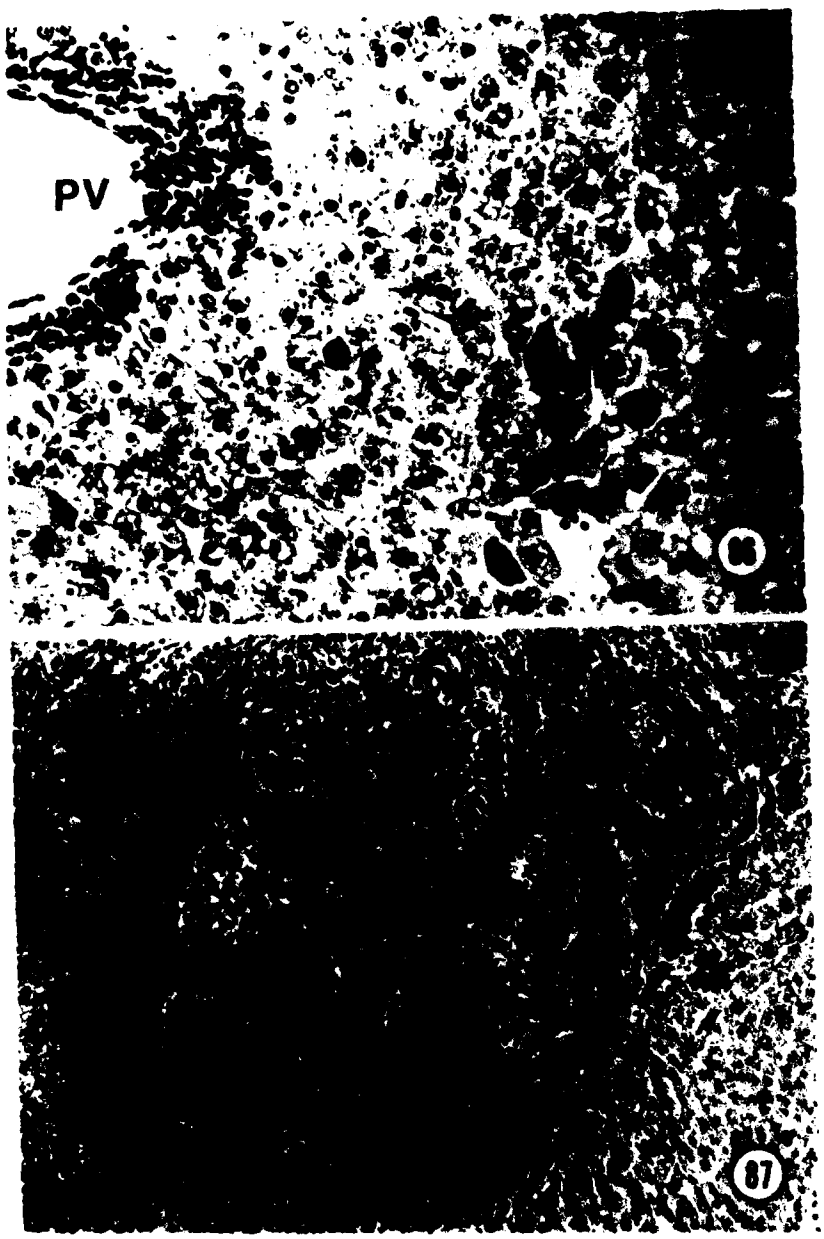

PV
C
87

Figure 88. Enlargement of figure 87. A nidus of round cells was located next to a small granuloma containing an ameba (arrow). 15 days, H&E, X250.


88

Nude Mouse Experiment

Twelve athymic nude mice and 17 normal littermates were infected with a dose of 1 x 10^6 HM-1 amebae to investigate the role of cell-mediated immunity in hepatic amebiasis. A preliminary experiment using C3H mice had shown that one million HM-1 trophozoites when inoculated intrahepatically were infective to mice although few amebae were found in the lesions. One group of six heterozygotes received 15 mg of cyclophosphamide per 100 g body weight 24 hours prior to infection. Six uninfected nu/nu and six nu/+ mice were controls. Except for the group of two infected nu/+ mice necropsied 18 days after infection, one-half of each group of six mice was necropsied nine days after infection and the remainder, at 18 days. Experimental data are tabulated in appendix 4 (tables 66-69).

Table 48 gives the mean number and size of amebic liver lesions in the infected groups. Heterozygous mice treated with cyclophosphamide before inoculation of amebae tended to have more lesions and larger ones than the other animals, but none of the differences was statistically significant.

The mice were weighed just prior to experimental treatment so that initial body weight could be used as a covariant for liver and spleen weight and body weight at the time of necropsy (table 49). There was no significant

variation among treatment groups or due to time after infection when liver weight and final body weight were adjusted for initial body weight. Table 50 shows the analysis of variance in spleen weight with orthogonal contrasts for the treatments. There was significant variation among treatment groups but no difference between nude and heterozygous mice. Also, uninfected controls did not differ significantly from athymic mice that received HM-1 amebae. The significant variation resided in the difference between uninfected and infected heterozygotes and between infected heterozygotes that received only amebae and those that had received CY prior to infection. There was a significant time effect and a treatment x time interaction. This interaction was no longer significant when spleen weight was adjusted for initial body weight (table 51). The significant difference among treatment groups was still present, however, as was a difference due to time after infection.

Hematology values for the mice just prior to the experiment facilitated analysis of covariance in the blood picture at the time of necropsy. After adjustment, none of the variables in the hematology of the mice showed any significant variation due to treatment or time.

Gross pathology and histopathology of the liver were similar in athymic and heterozygous mice. More lesions were found beneath the capsule of the liver than in the parenchyma, particularly 18 days after infection. Figure 89 shows normal histology of the liver in either heterozygous or nude mice.

Nine days after infection amebic lesions consisted of a granulomatous reaction surrounding a necrotic core (figure 90). A few mononuclear cells plus large numbers of PMN and many eosinophils (figure 91) infiltrated the edge of lesions where an occasional multinucleated giant cell (figure 92) was seen. Amebae were seen infrequently in lesions with intense acute inflammation and were never seen outside necrotic areas. Figure 93 shows a well-defined granuloma of nine days' duration in a CY-treated heterozygote.

At 18 days nu/nu and nu/+ mice usually had small numbers of amebae in the lesions while CY-treated mice had none. Figure 94 shows a large granulomatous lesion in a nude mouse that contained several identifiable amebae (figure 95). The amount of fibrosis in most lesions from the CY group indicated healing (figure 96).

One of the nu/+ mice treated with cyclophosphamide developed an amebic lesion in the left lung (figure 97). When the animal was necropsied nine days after infection,

the lesion measured 2 mm in diameter and contained apparently viable amebae. The histological appearance (figure 98) was the familiar granulomatous reaction seen in the liver. Amebae could have been inadvertently injected into the lung when the mouse was infected. This explanation seems more plausible than spread of amebae from the liver to the lung because no adhesions or other pathological changes were seen elsewhere in the body.

In general, athymic and heterozygous mice responded similarly to amebae. The mice were able to deal effectively with the infection by means of a granulomatous response and walling off the injury with fibrous tissue. Pathogenic HM-1 amebae were unable to reproduce in large numbers as they had in hamsters, so the mice were generally unsuitable as hosts. Cyclophosphamide seemed to have little effect on the course of amebic infection in the mice, but the absence of amebae in histological sections of 18-day lesions suggested that CY inhibited reproduction of the amebae.

TABLE 48

MEAN NUMBER AND SIZE OF LIVER LESIONS IN nu/nu AND nu/+ MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

	9 Days				18 Days			
	No.	Size of Largest Lesion			No.	Size of Largest Lesion		
Treatment Group*	Liver Lesions	Length (mm)	Width (mm)	Depth (mm)	Liver Lesions	Length (mm)	Width (mm)	Depth (mm)
nu/nu HM-1	4.67	2.67	1.67	1.67	3.67	3.67	2.00	2.00
nu/+ HM-1	6.67	2.83	1.27	1.27	1.50	2.00	1.00	1.00
nu/+ CY HM-1	9.00	3.93	2.60	2.60	6.33	3.40	2.00	1.50

NOTE: Uninfected control animals were not included.

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide.

TABLE 49

MEAN ORGAN WEIGHTS AND BODY WEIGHT OF nu/nu AND nu/+ MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

	9 Days				18 Days			
Treatment Group*	Liver Weight (g)	Spleen Weight (mg)	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (g)	Spleen Weight (mg)	Initial Body Weight (g)	Final Body Weight (g)
nu/nu control	1.763	114.87	30.43	28.97	1.667	150.00	27.33	25.53
nu/nu HM-1	1.777	209.67	23.87	25.37	1.827	125.80	21.23	26.20
nu/+ control	1.623	132.73	28.20	28.53	1.997	123.00	37.77	35.13
nu/+ HM-1	2.027	218.60	34.73	34.73	2.045	125.00	35.25	32.10
nu/+ CY HM-1	1.947	264.67	34.37	32.47	1.737	186.60	31.83	28.23

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide.

TABLE 50

ANALYSIS OF VARIANCE IN SPLEEN WEIGHT OF UNINFECTED CONTROLS AND nu/nu AND nu/+ MICE INOCULATED INTRAHEPATICALLY WITH ONE MILLION HM-1 TROPHOZOITES

Source of Variation	SS	d.f.	MS	F	P
Treatment group	35738.88	4	9384.59	4.9545	.007
nu/nu vs nu/+	2405.00	1	2405.00	1.2697	.274
nu/nu control vs nu/nu HM-1	3738.27	1	3738.27	1.9736	.176
nu/+ control vs nu/+ infected*	15344.04	1	15344.04	8.1008	.010
nu/+ HM-1 vs nu/+ CY HM-1	16051.07	1	16051.07	8.4741	.009
Time	23703.35	1	23703.35	12.5140	.002
Treatment x time interaction	33914.46	4	8478.62	4.4762	.010
Within cells	35988.64	19	1894.14		

* Both nu/+ HM-1 and nu/+ CY HM-1 groups are included in the category of nu/+ infected.

TABLE 51

ANALYSIS OF COVARIANCE OF SPLEEN WEIGHT WITH INITIAL BODY WEIGHT OF UNINFECTED CONTROLS AND *nu/nu* AND *nu/+* MICE INOCULATED INTRAHEPATICALLY WITH ONE MILLION HM-1 TROPHOZOITES

Source of Variation	SS	d.f.	MS	F	P
Initial body weight	3059.043	1	3059.043	1.586	.224
Treatment group	36476.070	4	9119.016	4.729	.009
Time	14503.617	1	14503.617	7.521	.013
Treatment x time interaction	17419.602	4	4354.898	2.258	.103
Error	34711.313	18	1928.406		

Figure 89. Normal liver in an athymic nude mouse. H&E, X250.

Figure 90. Granulomatous reaction to amebic necrosis in an athymic mouse nine days after intrahepatic inoculation with one million HM-1 trophozoites. H&E, X130.

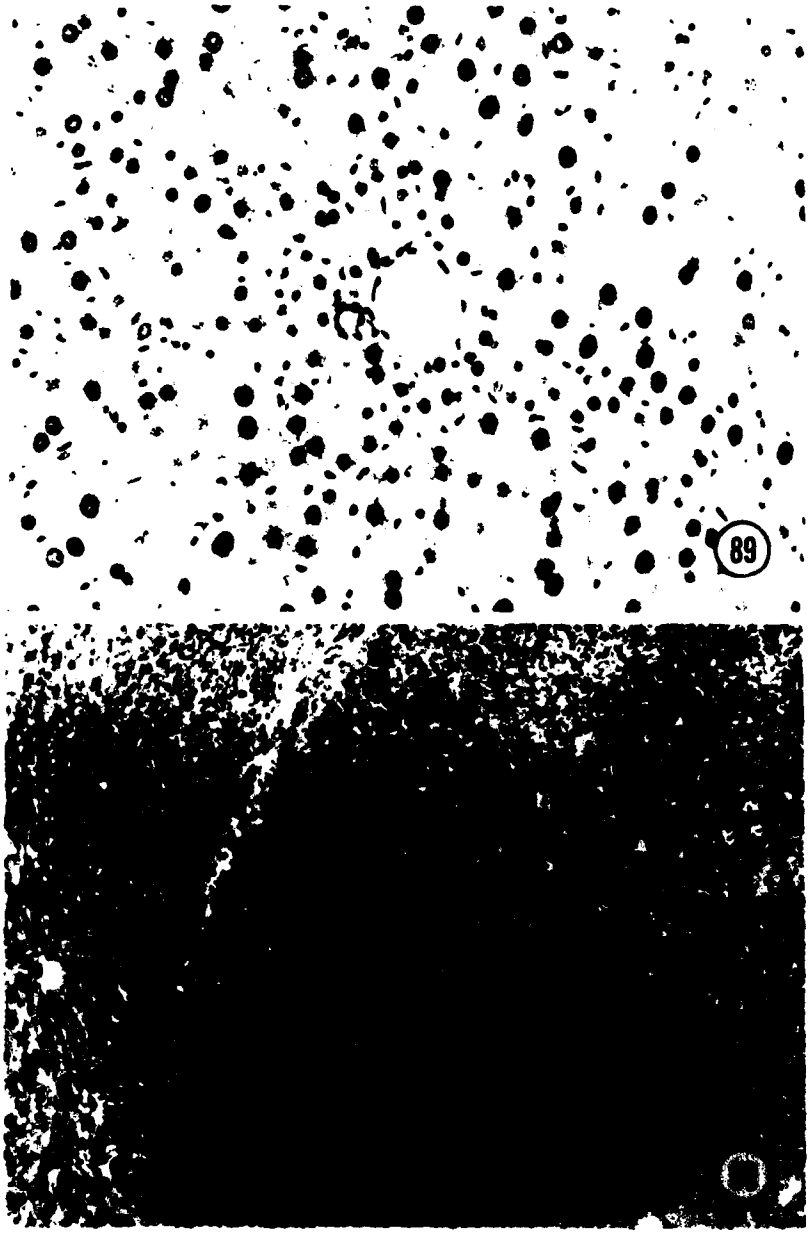

89

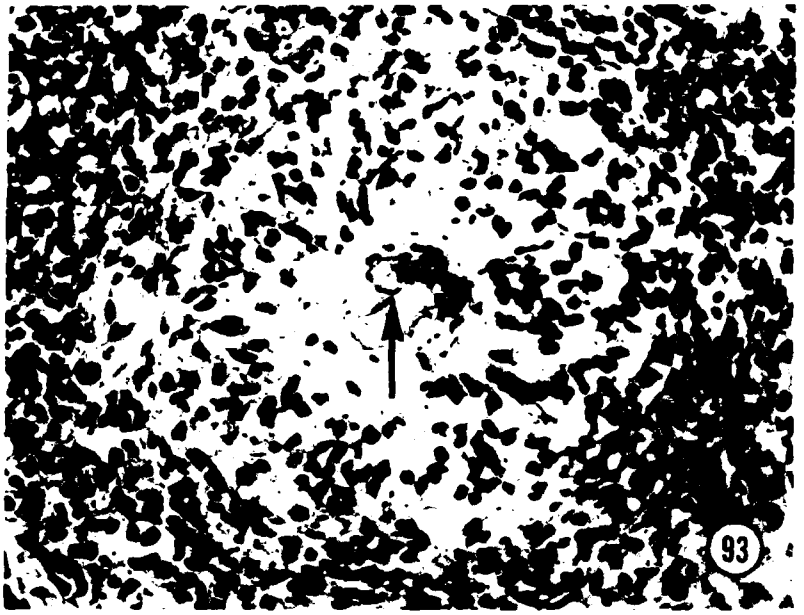
Figure 91. Mixed inflammatory cells at the edge of an amebic lesion (upper left) in a heterozygous mouse that was infected with one million HM-1 trophozoites nine days earlier. Mitotic figure at arrow. Eosinophils were numerous in this tissue section. H&E, X400.

Figure 92. Multinucleated giant cell (arrow) at the edge of an amebic lesion in a heterozygous mouse. 9 days, H&E, X400.

92

Figure 93. Granulomatous reaction to amebae (arrow) in the liver of a heterozygous mouse that received cyclophosphamide 24 h prior to infection. 9 days, H&E, X400.

Figure 94. Large granulomatous lesion on the surface of the liver from an athymic mouse 18 days after intrahepatic injection with one million HM-1 amebae. H&E, X40.


93


94

Figure 95. Enlargement of figure 94. Several amebae (arrow) were seen in this lesion. 18 days, H&E, X400.

Figure 96. Healing granuloma in the liver of a CY-treated heterozygous mouse 18 days after infection with one million HM-1 amebae. H&E, X100.

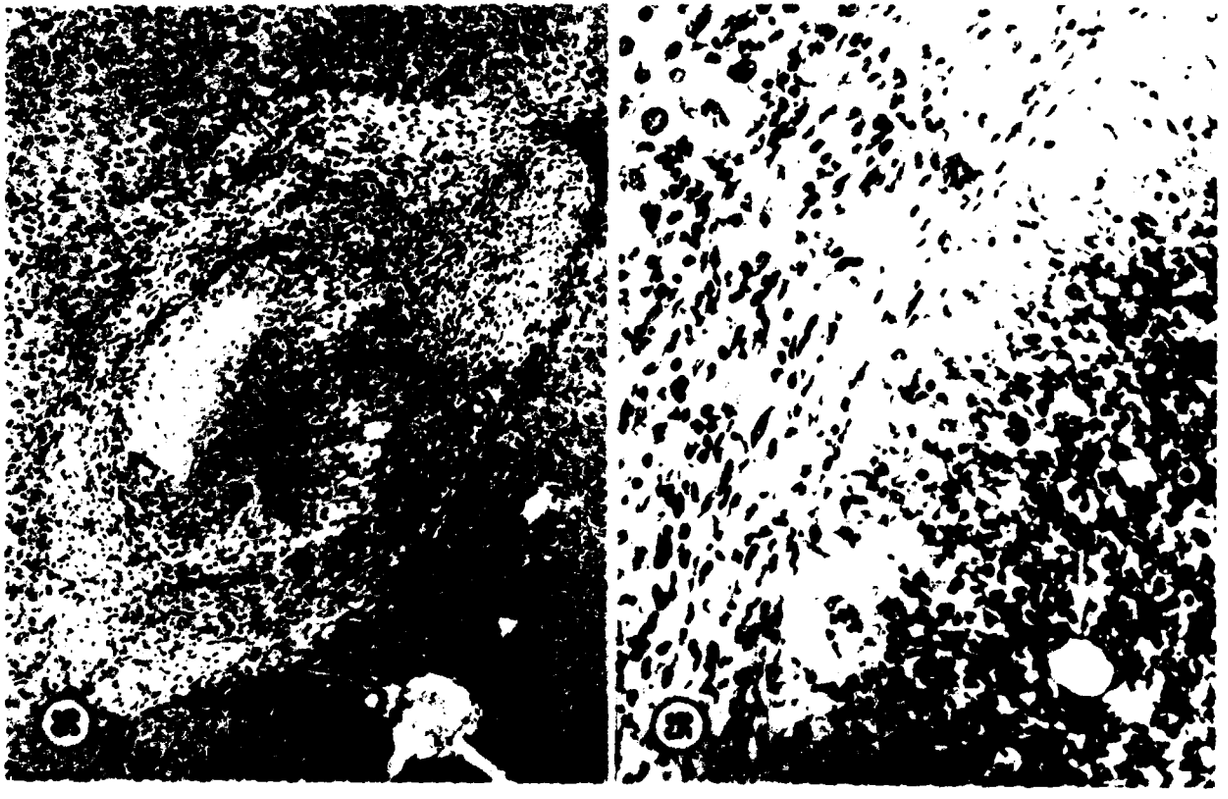


Figure 97. Amebic granuloma of the lung in a heterozygous mouse. This animal had been treated with cyclophosphamide 24 h prior to infection with one million HM-1 trophozoites. 9 days, H&E, X40.

Figure 98. Enlargement of figure 97. Granulomatous reaction to amebae (A) in the lung of a CY-treated heterozygous mouse. Notice margination of leukocytes (arrowhead) in the blood vessel at left. 9 days, H&E, X250.

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IV. DISCUSSION

Inbred LHC/Lak hamsters proved to be an excellent model for hepatic amebiasis. The virulent HM-1 strain of Entamoeba histolytica produced pathological and pathophysiological changes in hamsters that were very similar to those that occur in amebic liver abscess in man. Being less virulent or nonpathogenic in hamsters, the other axenic strains of amebae were less suitable for further study, although strain 200:NIH provided an opportunity to study repair of amebic lesions. Only strain HM-1 at a dose of 315 thousand was capable of killing 80% of infected hamsters over a period of 48 days.

Direct comparison of hepatic amebiasis in the hamster model with amebic liver abscess is generally complicated by lack of information about duration of the disease process in man. Patients with ALA usually seek medical attention after becoming symptomatic and reaching a chronic stage of their disease; therefore, the opportunity to study acute hepatic amebiasis has already been lost. This point emphasizes the importance of having an animal model to allow sampling at various stages of disease.

Axenic HM-1 amebae caused considerable damage when they were injected into the portal vein of hamsters. The liver became enlarged within a week after infection, and the extent of hepatomegaly was dose related. Hepatomegaly is one of the most important signs in human amebic liver abscess (Councilman and Lafleur 1891, Ochsner and DeBakey 1943, Wilmot 1962, Sheehy et al. 1968, Barbour and Juniper 1972, Adams and MacLeod 1977b). Other workers have reported hepatomegaly in hamsters with experimental hepatic amebiasis (Diamond et al. 1974b, Ghadirian et al. 1980, Lushbaugh et al. 1980b).

In hamsters, amebic lesions became larger with time but fewer in number because small necrotic foci coalesced into large areas of caseous necrosis. Palmer (1938) concluded that nearby amebic abscesses coalesce to form larger ones in man. Several studies have mentioned a direct relationship between the dose of amebae and the extent of liver necrosis in hamsters (Diamond et al. 1973, Ghadirian and Meerovitch 1978a, Martínez-Palomo et al. 1980). Data from this study suggested a similar dose response for the size of lesions caused by axenic HM-1 and 200:NIH.

Splenomegaly has already been reported in hamsters with hepatic amebiasis (Gold et al. 1978, Ghadirian et al. 1980); therefore, it was not surprising to find enlarged spleens in

hamsters that received HM-1 amebae, especially at higher dose levels. At least seven days were necessary for splenomegaly to appear. This time coincided with antibody production and elevated gamma globulin in serum. Mention of splenomegaly with human ALA has escaped notice in the literature. Future clinical and epidemiological studies of this disease should look for splenomegaly.

Bos (1973) and Ghadirian and Meerovitch (1978a) attributed loss of body weight to severe liver necrosis in experimentally infected hamsters. Data from this study indicated that loss of body weight initially resulted from surgery and the trauma associated with it. All the animals including sham-operated controls suffered a dramatic drop in weight within three days but regained to their original body weight within 15 days. Hamsters with the most severe disease due to HM-1 infection had persistent weight loss after accounting for hepatomegaly. Weight loss is sometimes a clinical manifestation of ALA (Ochsner and DeBakey 1943, Sheehy et al. 1968).

Mild anemia is an important finding in patients with amebic liver abscess (Wilmot 1962, Mayet and Powell 1964, Barbour and Juniper 1972, Adams and MacLeod 1977b), and the degree of anemia is related to the duration of symptoms and size of the abscess (Mayet and Powell 1964). Hemoglobin

concentration is usually less than 12 (Adams and MacLeod 1977b) or 13 g/dl (Mayet and Powell 1964, Barbour and Juniper 1972). Ochsner and DeBakey (1943) found their ALA patients to have RBC counts between 1.95 and 4.975 million cells per cu mm whereas Sheehy et al. (1968) found the hematocrit in most of their patients to be less than 35%. The literature is silent on this subject with respect to animal models. The mean RBC count and microhematocrit for sham-operated controls in this study were 7.06 million cells per cu mm and 51.1%, respectively. In severely infected groups of hamsters, a mean RBC count of 5.56-6.24 million cells per cu mm and mean microhematocrit of 38.0-42.0% packed cell volume were indicative of mild anemia, which was associated with larger liver lesions in the HM-1 and 200:NIH groups. Therefore, the model used in this study mimicked the human disease.

Sir Leonard Rogers (1922) first associated leukocytosis with amebic liver abscess. Barbour and Juniper (1972) also felt that leukocytosis is part of the clinical picture for ALA and reported WBC counts greater than 20 thousand cells per cu mm in almost one-half of their patients. Other authors have considered ALA patients to have leukocytosis when the WBC count exceeds 10 thousand (Powell 1959b, Adams and MacLeod 1977b) or 15 thousand cells per cu mm

(Sheehy et al. 1968). Ochsner and DeBakey (1943) stated that the white cell count diminishes as amebic liver lesions become more chronic. In their patients, the leukocytosis was often attributed to neutrophils because the percentage of neutrophils ranged from 59 to 97% of the WBC count. In this study all dose levels of 200:NIH and the highest dose of HM-1 amebae caused leukocytosis within one week after infection, but the white cell count diminished to almost normal by 15 days as the lesions were becoming chronic. The extent of leukocytosis was dose related in the HM-1 and 200:NIH groups, and mean values were 6762 to 11 142 cells per cu mm. Sham-operated controls had a mean WBC count of 4658. In experimentally infected hamsters, neutrophils were primarily reponsible for the higher WBC counts. The dynamics of leukocytosis and the cells responsible for it in the model agreed with the human blood picture in amebic liver abscess.

Circulating basophils were seen only in hamsters that received HM-1 amebae. These granulocytes were tentatively identified on the basis of their basophilic granules stained by a Romanowsky stain. Neutrophils and eosinophils in the hamster had azurophilic and eosinophilic granules, respectively. Basophilia in ALA patients has not been mentioned, and Ochsner and DeBakey (1943) found no eosinophilia in

their patients. Basophils are an important source of vasoactive amines and thus are involved in immediate hypersensitivity (Bellanti 1978). In addition, histamine and 5-hydroxytryptamine cause increased vascular permeability by stimulating formation of endothelial gaps in venules, an early event in inflammation (Wilhelm 1973). Whether basophils are involved with the inflammatory process to amebic invasion of the liver in hamsters is open to speculation. Because the existence of basophils in hamsters has been disputed (Hoffman et al. 1968), axenic Entamoeba histolytica strain HM-1 could serve as an experimental model to study the question.

Hamsters with experimental hepatic amebiasis had normal amounts of total protein in their serum, but animals with the most severe disease caused by HM-1 amebae had inverted A:G ratios. This finding resulted from decreased amounts of albumin accompanied by a dramatic increase in gamma globulin. Very similar results from serum protein electrophoresis obtain in patients with amebic liver abscess (Powell 1959b, Kamat et al. 1968). Elevated gamma globulin in experimentally infected hamsters was indicative of an inflammatory response to infection with antibody production detected by complement fixation at seven days and later. Although beta globulin is usually normal in ALA patients, alpha globulins

may be elevated (Powell 1959b, Kamat et al. 1968). Alpha-1 and -2 globulins were not elevated in serum from infected hamsters. Elevated beta globulin associated with HM-1 strain may have resulted in part from the technical difficulty in electrophoretically separating beta globulin from a large gamma globulin peak in the same samples. Serum protein electrophoresis apparently has not been done with animal models of amebiasis in the past.

Few ALA patients show jaundice (Ochsner and DeBakey 1943, Wilmot 1962) or have abnormal levels of bilirubin in serum (Wilmot 1962, Powell 1959b). Similarly, bilirubin was normal in experimentally infected hamsters.

A variety of serum enzymes has been measured to characterize the pathophysiology of amebic liver abscess. Alkaline phosphatase is usually elevated in ALA patients (Sepúlveda et al. 1959, Salako 1967, Kamat et al. 1968, Barbour and Juniper 1972). Likewise in the hamster model, alkaline phosphatase reached extremely high levels as tissue necrosis became extensive by seven days and stayed elevated for the duration of the experiment. Aldolase was elevated by three days and remained so, but not to the extent of alkaline phosphatase. The transaminases and lactate dehydrogenase showed similar patterns of early high values returning to normal by 11 days after infection. Rapid

release of transaminases and LDH was associated with acute necrosis of the liver. Aspartate aminotransferase (Sheehy et al. 1968, Santhanagopalan et al. 1968) and alanine aminotransferase (Santhanagopalan et al. 1968) may be elevated in ALA patients. Elevated transaminases have been previously reported in hamsters with hepatic amebiasis (Raether et al. 1967).

The pathophysiology of experimental hepatic amebiasis in hamsters closely paralleled the findings in amebic liver abscess of man. Powell (1959b) recognized the usefulness of studying pathophysiological variables to characterize the host response to amebic infection. In the hamster model, there was a dramatic response to massive necrosis in the liver. As amebic lesions encroached on the liver, a spectrum of pathological changes took place. Compensating for loss of functional hepatic parenchyma, the liver enlarged as some of its functions became impaired. Less albumin was manufactured while large amounts of gamma globulin occurred in serum resulting in an inverted A:G ratio. Splenomegaly and antibody production were concomitant with rising gamma globulin. Animals became slightly anemic and developed leukocytosis due to large numbers of neutrophils. Extremely high levels of alkaline phosphatase in serum reflected the extent of tissue necrosis.

The pathology of hepatic amebiasis caused by axenic HM-1 amebae in hamsters was remarkably similar to classic descriptions of liver pathology in patients with ALA. Trophozoites were experimentally placed in the portal circulation where they became emboli that lodged in small branches of the portal vein. The amebae caused focal necrosis with little inflammation until nearby lesions began to coalesce into large multifocal ones. Rogers (1922) and Palmer (1938) described similar changes in early stages of amebic liver abscess.

As experimental lesions became larger, fibroblasts began to wall off the injured area where the predominant inflammatory cell type was the macrophage. Dystrophic calcification of necrotic debris was evident as hematophagous amebae set up secondary foci by direct extension and hematogenous spread. As the host walled off the injury, amebae were proliferating too rapidly and starting new lesions by invading normal tissue. Masses of trophozoites were often seen within a feeding zone between the necrotic center and adjacent normal parenchyma. Mononuclear infiltration of periportal areas preceded extramedullary hematopoiesis in liver and spleen.

Toward the end of the experiment, 15 days after infection, some lobes of the liver were completely occupied

by caseous necrosis. The granulomatous tissue reaction was surrounded by a fibrous capsule. Severe pericholangitis accompanied by accumulations of plasma cells and extramedullary hematopoiesis were common in areas of the liver distant from amebic lesions. Liquefaction of lesion contents was an unusual finding even in latter stages of the disease process.

Pathological changes in the hamster model of hepatic amebiasis were similar to those in amebic liver abscess. Accounts by Councilman and Lafleur (1891), Rogers (1922), Palmer (1938), Ochsner and DeBakey (1943) and Faust (1954) contained similar, but not identical, descriptions of pathologic changes. Two differences stand out. Amebic lesions in human liver usually become fluid filled, but this was an uncommon finding in two-week-old hamster lesions. Macrophages seemed relatively more important than neutrophils in the inflammatory response to severe amebic necrosis in hamsters whereas neutrophils are the predominant inflammatory cell associated with extensive necrosis in ALA (Faust 1954). However, Lushbaugh et al. (1980b) discovered that a substrain of axenically cultivated HM-1 E. histolytica passaged in hamster liver could produce fluid-filled lesions with a fibrous wall six weeks after infection. These lesions in the hamster model had lost their granulomatous

appearance. Then given the opportunity to study amebic liver lesions at comparable stages of the disease process in man and hamsters, one may find the differences between host responses to be negligible.

Ultrastructural changes in the liver of patients with ALA have not been described. In the hamster model, hepatocytes suffered damage to the cytoplasm before becoming completely necrotic. Glycogen was depleted from the cell. Rough endoplasmic reticulum exhibited fewer arrays and became dilated. Mitochondria became swollen and lost their typical architecture including cristae and granules. Parts of the host cytoplasm were almost devoid of organelles although the nucleus appeared less affected. Lowe and Maegraith (1970c) described similar changes in liver cells of hamsters infected with Entamoeba histolytica.

In summary, axenic strain HM-1 Entamoeba histolytica afforded the best opportunity for studying the pathophysiology and pathology of experimental hepatic amebiasis in hamsters. Strain 200:NIH amebae duplicated some of the early effects of HM-1 in the hamster model and provided an opportunity to study the repair process. Either the other two axenic strains of E. histolytica failed to become established in the hamster liver, or host defenses were able to ward off the less aggressive, nonpathogenic amebae at

doses used in the experiment. Therefore, HM-1 was the best choice for a model of hepatic amebiasis. The primary effect of this parasite on the liver was necrosis. The host mounted a granulomatous response with fibroplasia that partially contained the infection. A variety of measures to study the pathophysiology of this infection correlated well with the pathologic changes so obvious in the tissue. Once HM-1 amebae had gained a foothold, such a virulent organism overcame host defenses.

Because a wide variety of measures all pointed to great similarity between hepatic amebiasis in hamsters and amebic liver abscess in man, LHC/Lak inbred hamsters provided an excellent model for this disease when axenic HM-1 Entamoeba histolytica was used to infect the liver. Table 52 compares the pathology and pathophysiology of amebic liver disease in man with the hamster model. Again, the comparison is hindered by a paucity of information about duration of the disease in patients from whom the published data were obtained.

The effects of immunosuppression on hepatic amebiasis were studied in the hamster to learn more about the immunology of this disease. This experimental approach seemed valid because immunosuppression can exacerbate amebiasis in patients (Kanani and Knight 1969a, b; Bailenger et al.

TABLE 52

COMPARISON OF HEPATIC AMEBIASIS IN THE INBRED HAMSTER WITH AMEBIC LIVER ABSCESS IN MAN

Variable	Man	Hamster
Pathology		
Effect of ameba on tissue	necrosis	necrosis
Contents of lesion	usually fluid	usually solid
Cellular reaction	fibrotic	granulomatous
Chief inflammatory cell	neutrophil	macrophage
Hepatomegaly	usually	pronounced
Splenomegaly	no data	pronounced
Pathophysiology		
Weight loss	sometimes	usually
Anemia	usually mild	mild
Leukocytosis	diminished with chronicity	diminished with chronicity
Peripheral neutrophils	often increased	increased
Peripheral eosinophils	normal	normal
Total serum protein	normal	normal
A:G	inverted	inverted
Albumin	decreased	decreased
Alpha-1 globulin	increased	normal
Alpha-2 globulin	increased	normal
Beta globulin	usually normal	increased
Gamma globulin	increased	increased
Antibodies/*E. histolytica*	yes	yes
Bilirubin	normal	normal
Alanine aminotransferase	sometimes increased	early increase
Aspartate aminotransferase	sometimes increased	early increase
Lactate dehydrogenase	no data	early increase
Aldolase	no data	slightly increased
Alkaline phosphatase	increased	greatly increased

1972). For exacerbating the disease in hamsters, anti-thymocyte serum following neonatal thymectomy and ATS alone were more effective than antilymphocyte serum with or without neonatal thymectomy. The number of lesions at six days was greater in the ATS and TX + ATS groups, but the size of lesions was smaller in the immunosuppressed animals than in the NRS control group. Even so, the total amount of amebic necrosis in livers from the ATS and TX + ATS groups was undoubtedly greater than in the appropriate controls. Therefore, immunosuppression by ATS alone or in combination with neonatal thymectomy resulted in more severe disease.

Neonatal thymectomy enhanced the effect of ATS in depressing antibody production and peripheral lymphocytes. By itself, ATS markedly depleted T cells in the normal hamster spleen and depleted B cells as effectively as cyclophosphamide. Lewis et al. (1981) showed that neonatal thymectomy renders adult hamsters deficient in T cells as measured by response of blood lymphocytes to ConA; these animals are also susceptible to tumors induced by transformed cells. Then hamsters in the TX + ATS group were likely deficient in both T and B lymphocytes.

Ghadirian and Meerovitch (1981) used antiserum to hamster cells in conjunction with neonatal thymectomy to exacerbate hepatic amebiasis in LHC/Lak hamsters. They

inoculated pathogenic axenic amebae passaged through hamster liver into the liver parenchyma and weighed both primary and metastatic lesions 10 days later. Primary liver lesions and metastatic ones were significantly larger in animals treated with the antiserum following neonatal thymectomy. Unfortunately, no data were given to show the effects of the antiserum on T and B cell populations determined by mitogen stimulation, although the serum was said to be specific for T cells. Nevertheless, neonatal thymectomy followed by antithymocyte serum seemed to be the best method to exacerbate hepatic amebiasis in the hamsters. Necrotic lesions in these animals had less cellular infiltration; and some metastatic foci were found in spleen, kidneys, and mesenteric lymph nodes. In this study amebic lesions were not seen in those tissues, but infiltrating mononuclear cells were conspicuously absent from liver lesions in the TX + ATS group. Although intraportal inoculation allowed amebae to spread throughout the liver instead of being localized initially to one injection site, results with neonatal thymectomy and antithymocyte serum in this study were similar to those obtained by intrahepatic inoculation.

At first, cyclophosphamide seemed to reduce the pathological effects of amebae. A sufficiently high dose, 22.5 mg per 100 g body weight, was selected to lower the WBC

count without killing the animals. That dose of CY depleted both T and B cells in the spleen when stimulated with mitogens. The number and size of liver lesions were markedly reduced in CY-treated hamsters compared to controls. Another experiment confirmed these findings. However, cyclophosphamide seemed to have a deleterious effect on the reproduction of amebae in the liver. Instead of exacerbating the disease, cyclophosphamide had the opposite effect by slowing the growth of the parasite. A solution of cyclophosphamide in normal human serum kills trophozoites in vitro within 24 hours (Abioye 1972).

Wijesundera (1980) reported that nine days after intracecal inoculation with amebae, inbred C3H/mg mice treated with antilymphocyte serum developed amebic lesions in the liver. The hypothesis was that T cells protected untreated mice against amebic invasion of the liver. Because of deficiency in functional T cells, athymic nude mice provided a means to test this hypothesis. The hypothesis was more plausible in light of the granulomatous reaction in hamsters, a type of cellular response often associated with cellular immunity mediated by T lymphocytes (Unanue 1978). The results were disappointing, however. Athymic mice and their normal littermates seemed to contain pathogenic HM-1 amebae equally well by walling off the

injury with fibrosis. A granulomatous response surrounded necrotic foci where small numbers of amebae were found. Cyclophosphamide did not alter the course of infection in heterozygous mice but inhibited growth of the amebae.

Both athymic and heterozygous mice were poor hosts for the parasite. Other workers have attempted to use the mouse as a model for hepatic amebiasis but without much success (Westphal and Michel 1970, Gold and Kagan 1978). The advantages of an inbred mouse model for amebic liver abscess are so great, however, that efforts should continue to develop such a model. Finding a granulomatous amebic lesion in the lung of a mouse in this study has suggested the possibility of further experiments using axenic amebae or amebic antigens on particles that could be injected into the tail vein of the mouse. Then experiments with this model could explore immunological events in the formation of granulomas in the lung and compare the lesions with naturally occurring ones in human lung. Von Lichtenberg pioneered this type of experiment for studying the granulomatous reaction to schistosome egg antigens (Von Lichtenberg 1962, Von Lichtenberg et al. 1971).

The pathogenesis of hepatic amebiasis remains enigmatic, but one can suggest an hypothesis about the pathologic process in the liver of experimentally infected hamsters. To

develop the hypothesis, one must assume that immunological events of inflammation in the hamster conform to current knowledge about inflammation in general.

Early events begin with amebae arriving in the liver as emboli in the portal circulation. Bacteria in the gut may affect the pathogenicity of amebae that become extra-intestinal. In man, soluble antigens and specific antibodies probably precede arrival of amebae in the liver; but neither of these was necessary for amebic liver lesions in the hamster. After amebae reach the liver, the host-parasite relationship is a delicate balance that can tip in favor of the host or parasite depending upon the success of host defenses and pathogenicity of the parasite.

Earliest lesions in the hamster resembled infarcts as they do in man (Palmer 1938). This event alone may initiate thrombosis and hence inflammation, or thrombosis may occur later as a result of inflammation (Rodman 1973). Amebae surely injure the endothelium to begin a complex series of immunological events that may lead to inflammation and further injury or to repair.

Entamoeba histolytica seems well equipped to promote inflammation. This parasite may produce prostaglandins in vitro (Das and Padma 1977), and some prostaglandins increase

vascular permeability as well as disrupt mast cells (Wilhelm 1973). When they degranulate, mast cells release histamine and 5-hydroxytryptamine, potent vasoactive amines (Bloom 1974). Mast cells were normally found in connective tissue of portal tracts in the hamster, so they were in the vicinity of the initial injury. In addition, E. histolytica can activate complement by the alternate pathway (Ortiz-Ortiz et al. 1978, Huldt et al. 1979). Derivatives of complement are potent chemotactic factors, especially for polymorphonuclear leukocytes and mononuclear cells (Nelson 1974). Extravascular chemotactic effects coupled with increased vascular permeability set the stage for chemotaxis of leukocytes and their emigration to the injury. Whether the protease activity of amebic cytotoxin (Lushbaugh et al. 1981) can explain early necrosis remains to be shown, but the necrotic process is central to the inflammatory process in the liver. Takeuchi et al. (1977) postulated that disintegrating neutrophils are largely responsible for histolysis of the mucosa in amebic dysentery.

As macrophages appear at the injury, the lesion takes on a granulomatous appearance. In several other infectious diseases such as tuberculosis, leprosy, histoplasmosis, and schistosomiasis, granulomatous inflammation is mediated by thymus-derived lymphocytes (Spector 1974, Unanue 1978). By

analogy one can hypothesize that cell-mediated immunity is responsible for the cellular reaction in amebic granulomas. Antigen-sensitized T cells activate macrophages by means of lymphokines and induce them to remain at the site of inflammation through the influence of migration inhibitory factor (Dvorak 1974). Spector (1974) considered macrophages derived from circulating monocytes to be the principal inflammatory cells in chronic granulomatous inflammation. Besides lymphocytes, fibroblasts and eosinophils are also prominent in a granulomatous response (Spector 1974). Both mast cells and lymphocytes produce eosinophil chemotactic factors; eosinophils in turn produce substances that help modulate the effects of mast cells (Goetzl and Austen 1977). Fibroplasia and synthesis of collagen are important components of tissue repair (Dumont 1974).

If the irritant that caused a granuloma persists, chronic inflammation is the result (Spector 1974). Irritants may persist because macrophages have incompletely degraded them. However, disappearance of the irritant resolves the granulomatous reaction (Spector 1974). In this study persistence of the parasite was associated with chronic granulomatous inflammation while disappearance of the parasite was associated with repair.

A variety of host effector mechanisms could explain disappearance of amebae. For example, antibody and complement lyse amebae in vitro (Guerrero-Alcázar et al. 1972, Sepúlveda et al. 1974, Huldt et al. 1979). This mechanism is probably not important as a host defense because animals and human beings with severe amebic liver disease often have high titers of antibody. Furthermore, E. histolytica can internalize and degrade antibody bound to its surface (Aust-Kettis et al. 1981). Then an effector cell may damage and kill amebae in the host. Cytotoxic T lymphocytes may act alone or in cooperation with macrophages (McGregor and Mackaness 1974) to destroy the parasite. The phagocytic properties of macrophages and neutrophils may be important also. Finally, eosinophils produce superoxide (Goetzl and Austen 1977), which may be lethal to amebae.

The immunological events during amebic invasion of the liver need to be studied further in the hamster model now that validity of the model has been established. Although amebic liver lesions in man and the hamster may have a different histological appearance, studying the amebic granuloma in the model may help elucidate the pathogenesis of amebic liver abscess in man. It seems likely that macrophages and cell-mediated immunity are central to the formation of amebic granulomas in the animal model.

Recently, an in vitro model of the schistosome egg granuloma shows promise for studying granuloma formation under simpler conditions than those in the host (Bentley et al. 1982). Perhaps similar in vitro experiments can be done with the amebic granuloma.

V. CONCLUSIONS

The inbred LHC/Lak hamster proved to be an excellent model for hepatic amebiasis. Entamoeba histolytica caused pathological and pathophysiological changes in the model similar to those in amebic liver abscess of man. Judicious selection of axenic strain and dose of amebae produced a spectrum of disease in the model ranging from self limiting injury to severe, sometimes fatal disease. The most pathogenic parasite used in this study, strain HM-1, was the overall best choice; but strain 200:NIH was useful for studying repair of amebic lesions in the liver. Inbred hamsters infected with axenic amebae via the portal vein satisfied important criteria for an animal model; namely, the disease process in a model must be analogous to that in man and should reflect the human spectrum of disease.

Table 52 compares experimental hepatic amebiasis in the hamster with published descriptions of amebic liver abscess in man. The most notable dissimilarity was the cellular reaction to amebic invasion of the liver. Although neutrophils are the predominant inflammatory cells associated with the fibrotic response about fluid-filled absesses in man, macrophages were the chief inflammatory cells in

granulomatous inflammation to solid lesions in hamsters. However, this apparent difference in pathology may result from comparing lesions in the two hosts at disparate times during the natural course of the disease. The primary effect of E. histolytica on host tissue was necrosis. Weight loss and hepatomegaly were part of the clinical picture in both hosts; enlargement of the spleen was also pronounced in hamsters.

Comparison of a number of pathophysiological variables revealed close similarity of hepatic amebiasis in the hamster and man. For example, the blood pictures were very similar. Mild anemia was associated with leukocytosis due to increased numbers of neutrophils, but the leukocytosis diminished as lesions became chronic. The number of peripheral eosinophils was normal. Although total serum protein was normal, the A:G ratio was inverted because of less albumin and elevated levels of globulins in serum. As in amebic liver abscess, gamma globulin was increased in severe hepatic amebiasis of hamsters. Both man and hamster produce antibodies to E. histolytica when they have amebic lesions in the liver. Patients with amebic liver abscess are rarely jaundiced and have normal levels of bilirubin as did experimentally infected hamsters. Alkaline phosphatase was the most important liver enzyme measured in the experimental

disease because the amount of this enzyme reflected the extent of liver necrosis. In patients with hepatic amebiasis, this enzyme is more likely to be elevated than transaminases. On the whole, there was remarkable similarity between experimental hepatic amebiasis in the inbred hamster and amebic liver abscess in man.

Antithymocyte serum following neonatal thymectomy exacerbated amebic lesions in hamsters better than other means of immunosuppression including cyclophosphamide, which had a deleterious effect on amebae. This result and the known involvement of T cells in granulomatous inflammation suggested that cell-mediated immunity was important in the immunology of experimental hepatic amebiasis. To test this hypothesis, athymic nude mice and their normal littermates were infected intrahepatically with pathogenic HM-1 amebae. The experiment with mice was inconclusive because they were poor hosts for the parasite and T cell deficient mice contained the infection as well as heterozygous animals. Without a murine model, the LHC/Lak inbred hamster offers the best opportunity to study cell-mediated immunity in hepatic amebiasis.

APPENDIX 1

REVIEW OF LITERATURE

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APPENDIX 1

REVIEW OF LITERATURE

In this appendix, more information than in the Introduction is given about Entamoeba histolytica and amebiasis. Topics include the epidemiology of amebiasis, intestinal amebiasis, amebic hepatitis, and other aspects of immunology besides immunosuppression. Finally, other animal models besides the hamster are reviewed.

Morphology of *Entamoeba histolytica*

The following morphologic description by light microscopy of E. histolytica was taken essentially from Levine (1973). Two different morphologic stages occur in the life cycle of E. histolytica: the feeding trophozoite stage and the resting cyst stage. Trophozoites when rounded measure 20-30 μm in diameter and exhibit a characteristic unidirectional, determined ameboid motion leading with a single pseudopodium at the anterior end. Although the nucleus is usually indistinct in living trophozoites, it has a very characteristic morphology when it is appropriately stained for light microscopy. The endosome or karyosome is small and often centric. The peripheral

chromatin consists of fine uniform particles evenly distributed about the nuclear membrane, and scattered chromatin granules may occur between the peripheral chromatin and endosome. The cytoplasm of the trophozoite in a stained preparation has a smooth appearance but may contain food vacuoles, some of which may contain ingested erythrocytes if the ameba is tissue invasive. Cysts are 10-20 µm in diameter and have four nuclei when mature. They propagate the life cycle and can withstand environmental conditions.

Electron microscopy has added new dimension to the morphology of Entamoeba histolytica. The fine structure of trophozoites is not appreciably different regardless of their source (Rosenbaum and Wittner 1970, El-Hashimi and Pittman 1970, Feria-Velasco and Treviño 1972).

Reviewing the ultrastructure of trophozoites, Ludvík and Shipstone (1970) described the plasmalemma as a typical trilaminar unit membrane 12 000 nm thick (Griffin and Juniper 1971) with scattered subpellicular dark bodies. A fuzzy coat on the outer surface of the plasma membrane occurs on trophozoites from human colon (El-Hashimi and Pittman 1970) and human liver (Proctor 1976). Lushbaugh and Miller (1974) demonstrated that the fuzzy coat or glycocalyx of axenically

cultured amebae is much more uniform and compact than that of monoxenic or in vivo trophozoites.

When pseudopodia are extended, they are generally devoid of organelles (Ludvík and Shipstone 1970, Proctor and Gregory 1972). Otherwise, differentiation between ectoplasm and endoplasm by electron microscopy is usually difficult (Ludvík and Shipstone 1970). Lowe and Maegraith (1970c) saw ectoplasm in occasional trophozoites in situ. During the process of phagocytosis E. histolytica trophozoites make close contact with the prey by means of an adhesive region, the phagocollar (Westphal et al. 1972), which generally lacks inclusions (Griffin and Juniper 1971) and serves as the invagination channel. McCaul (1977) found that micropseudopodia line the walls of the phagocytic channel and fuse at the tips to form membrane-bound vacuoles. Vesicles containing ingested material may be seen in the cytoplasm near the end of the channel and apparently fuse with lysosomes. Trophozoites can ingest cells with intact plasma membranes (McCaul 1977).

Perhaps the most-studied surface feature of trophozoites is the so-called surface active lysosome (Eaton et al. 1969, 1970). By demonstrating the need for contact between amebae and lysed tissue culture cells, Eaton et al. (1969, 1970) believed the cup-shaped organelle with its

frond-like trigger is related to pathogenicity of E. histolytica. Although several authors have since made similar observations by transmission electron microscopy, Deas and Miller (1977) avoided using the term lysosome for the subplasmalemmal vacuole, which has dendritic extensions (the trigger) contiguous with the plasmalemma. Lushbaugh and Pittman (1979) interpreted the surface active lysosomes with a trigger and dendritic plasmalemmal extensions as parts of filopodia whose cytoplasm is continuous with the cell cytoplasm. They observed swellings along the length or at the tips of filopodia, which sometimes measure over 100 μm long.

Several cytoplasmic organelles are described but mitochondria are absent (Miller et al. 1961). Eaton et al. (1970) and Proctor and Gregory (1972) reported stacked membranes resembling Golgi apparatus, yet Feria-Velasco and Treviño (1972) saw similar membranes only in amebae incubated with emetine or metronidazole. Various kinds of vacuoles occur in the cytoplasm with food vacuoles being most numerous (Ludvík and Shipstone 1970). The limiting membrane of vacuoles is ultrastructurally identical to plasmalemma (Miller et al. 1961). Myelin forms or whorls commonly occur in food vacuoles (Lowe and Maegraith 1970a, b), each of which typically contains only one type of food

(Fletcher et al. 1962). Lowe and Maegraith (1970b) described intracellular digestion of erythrocytes. Acid phosphatase activity occurs in food vacuoles (Rosenbaum and Wittner 1970), lysosomes (Eaton et al. 1970, Lushbaugh et al. 1972), and endoplasmic reticulum as well as on surface blebs (Lushbaugh et al. 1972). Glycogen is a prominent feature in cytoplasm and often occurs as rosettes (El-Hashimi and Pittman 1970). Griffin and Juniper (1971) reported microfilaments, which may be involved in cytoplasmic streaming (Michel and Schupp 1974) or phagocytosis but not in locomotion (Michel and Schupp 1976). Several authors saw microtubules (Ludvík and Shipstone 1970, Rosenbaum and Wittner 1970, Proctor and Gregory 1972). Endoplasmic reticulum consists of fine tubules and small lacunae often lacking ribosomes (Griffin and Juniper 1971, Proctor and Gregory 1973b) but sometimes surrounded by them (Ludvík and Shipstone 1970, Lowe and Maegraith 1970b).

Ribosomes in E. histolytica trophozoites occur as single helices of particles approximately 18 000 nm in diameter or as stacked helices in the form of crystalline structures (Rosenbaum and Wittner 1970). Siddiqui and Rudzinska (1965) had already shown that similar helical ribonucleoprotein bodies correspond to chromatoid bodies in trophozoites of Entamoeba invadens. Although chromatoid

bodies commonly occur in Entamoeba cysts, they are also seen in HK-9 and 200:NIH trophozoites of E. histolytica by light microscopy (Proctor and Gregory 1973b).

Miller and Swartzwelder (1960) raised the possibility of viruses in E. histolytica when they published an electron micrograph showing virus-like particles in the cytoplasm of a trophozoite. Rosettes of electron dense, cylindrical bodies (or single ones) occur in the cytoplasm of axenic trophozoites (Feria-Velasco and Treviño 1972) as well as trophozoites in human colon (El-Hashimi and Pittman 1970) or liver (Proctor 1976). Mattern et al. (1976) found that the cytoplasmic bars differentiate into T bars, possibly analogous to bacteriophage. Transmissible viruses occur in all 10 axenic strains of E. histolytica examined thus far (Diamond et al. 1976). At least three types of amebal viruses are now documented: icosahedral virus in the cytoplasm, filamentous virus in the nucleus, and a beaded virus also in the nucleus (Diamond et al. 1972, Mattern et al. 1977).

The nucleus has a nuclear membrane consisting of a typical trilaminar unit membrane perforated by numerous pores (Ludvík and Shipstone 1970). Chromatin is dispersed in irregular clusters beneath the nuclear membrane. The endosome (karyosome) consists of small electron dense

clusters (Ludvík and Shipstone 1970). In E. histolytica-like ameba of Laredo strain a microtubule organizing center generates microtubules of the mitotic spindle during the entirely intranuclear mitotic process (Gicquaud 1979). Button-like bodies and vesicles occur in the nucleus but their function is unknown (Ludvík and Shipstone 1970). A paranuclear body may be seen in the cytoplasm near the nucleus (Proctor and Gregory 1972, 1973b; Lushbaugh and Miller 1974).

Lowe and Maegraith (1970a) described the fine structure of a "precystic" ameba from axenic culture of HK-9. Its cytoplasmic inclusions are pushed toward the periphery of the cell leaving a nearly vacant space in the center. Prominent ribonucleoprotein bodies vary in size. Otherwise, the fine structure of the precystic ameba is similar to the trophozoite.

Proctor and Gregory (1973a) studied the ultrastructure of E. histolytica cysts from human feces. The cyst wall has an outer fuzzy layer and a denser inner fibrous layer. A double membrane bounding the cytoplasm lies just beneath the inner layer of the cell wall. Cytoplasmic organelles and nuclei of cysts are ultrastructurally the same as in trophozoites. Glycogen is evenly distributed in the cytoplasm or else occurs in large crystalline masses unbound by membranes.

Epidemiology of Amebiasis

The epidemiology of amebiasis is essentially that of a disease contracted through fecal contamination of food or drink, or by person-to-person transmission (Spencer et al. 1977). The resistant cyst stage is ingested, and infection is established when cysts excyst in the small intestine of man. Any vehicle for carrying the cysts to the mouth is a suitable source of infection. In epidemics of amebiasis the cysts are usually water borne (Hardy and Spector 1935, LeMaistre et al. 1956). Two other modes of transmission have come to light in recent years. Istre et al. (1982) reported an outbreak of amebiasis associated with an improperly disinfected machine used for colonic irrigation at a chiropractic clinic in Colorado. Venereal transmission of amebiasis probably occurs among heterosexuals and homosexuals (Most 1968, Kean 1976, Kean et al. 1979, Pomerantz et al. 1980).

Elsdon-Dew (1968) published the most comprehensive review on the geographic distribution and prevalence of amebiasis to date. His paper grew out of a 1964 report (Elsdon-Dew 1964) prepared for the World Health Organization, which estimated the world prevalence of infection with Entamoeba histolytica at 10% of the world's population in 1969 (World Health Organization 1969). The prevalence of

disease due to this ameba is much less, however. The notion that amebiasis is a tropical disease is easily dispelled when one considers that the organism was originally described from St. Petersburg, Russia (Lösch 1875), and that prevalence rates of infection between 16% and 58% have been encountered in arctic Greenland (Babbott et al. 1961) and Saskatchewan, Canada (Meerovitch and Eaton 1965). Prevalence of infection is usually higher in the tropics of Africa and Asia where the disease is a significant cause of morbidity and mortality. In providing tabulated prevalence rates from numerous sources throughout the world, Elsdon-Dew (1968) was justifiably cautious about making comparisons among different investigators because of wide variation in the methods used to make the diagnosis and even disagreements about the case definition. Few authors had bothered to distinguish between Entamoeba histolytica and E. hartmanni when they determined the prevalence of amebiasis.

A variety of factors contribute to high prevalence of amebiasis, but less is known about conditions that are conducive to high prevalence of amebic disease. Person-to-person transmission in families is associated with high prevalence of amebiasis in several populations: Nigerians (Nnochiri 1965), people in India (Mathur and Kaur 1972), Canadian Indians (Eaton 1968), American blacks (Spencer et

al. 1976), and Spanish-Americans (Spencer et al. 1977). Crowding in a closed population such as that of Easter Island can lead to a prevalence rate of 24% with little disease (Meerovitch and Gibbs 1969, Meerovitch and Healy 1976). Healy et al. (1969) observed a similar situation among Cherokee school children. Two studies reported an association between low socioeconomic status and high prevalence of infection (Brooke et al. 1963, Spencer et al. 1976). Institutionalized populations, particularly the mentally retarded, have a high risk of amebic infection (Brooke 1963, Schultz 1974). In some populations the prevalence of infection with Entamoeba histolytica increases with age as in Nigeria (Oyerinde et al. 1977) and in Gambia (Bray and Harris 1977b). In other populations, the prevalence rate does not change much with age as on Easter Island (Meerovitch and Gibbs 1969) and among blacks in Arkansas (Spencer et al. 1976). The presence of infected food handlers in the household is associated with higher prevalence of infection in India (Mathur and Kaur 1972) but not in the United States (Brooke et al. 1955). Africans in Durban, South Africa, have a higher prevalence and greater severity of invasive amebiasis than Indians and Europeans (Freedman 1958). The seemingly racial difference in prevalence is explained on the basis of differences in

hygiene and diet complicated by rapid person-to-person transmission of the parasite (Freedman 1958, Powell et al. 1966a).

Somewhat surprisingly, the epidemiology of amebiasis in the United States does not present a clear picture because the data are no easier to interpret than data from other parts of the world. Schultz (1974) pointed out that the United States lacks a reporting system for amebiasis similar to the one on the incidence of malaria. He reiterated that few laboratories distinguish between Entamoeba histolytica and E. hartmanni, yet E. histolytica may account for one- to three-fourths of the combined prevalence of the two species in the United States (Burrows 1961). Juniper (1971) aptly pointed out that surveys of amebiasis prevalence have been largely based on samples that are not representative of the U.S. population at large. Primarily due to laboratory error, serious problems of over diagnosis in populations and under diagnosis in individual patients still exist in this country (Schultz 1974, Krogstad et al. 1978b).

Amebiasis is endemic in the United States and imported cases are not infrequent (Most 1974). In 1961 Burrows estimated the prevalence of infection with E. histolytica at less than 5% in the continental United States. Krogstad et al. (1978b) believed that figure is too high now based on

more recent data. Whether there has been an actual decline in prevalence of amebiasis is open to debate.

Certain subpopulations in the United States have higher prevalence of amebic infection than the general population. Otto (1973) reviewed data from stool surveys and reported high rates of infection among Indians (2-33% prevalence) and mental patients (1-40%). Recent immigrants or travelers from tropical countries also have higher prevalence (Brooke 1964a). In the United States prevalence of amebic infection is higher in rural than in urban areas and higher in the south than in the north (Brooke 1964a, b; Otto 1973).

Krogstad et al. (1978b) believed that serology of amebiasis will become increasingly important for estimating prevalence because the microscopic diagnosis of E. histolytica is fraught with difficulty. Indirect hemagglutination is useful in estimating the prevalence of invasive amebiasis in populations (Healy 1976). Seroepidemiological surveys on Indian reservations show significant IHA titers in 2-24% of the sampled populations. IHA titers above 1:64 occur in 11-45% of patients in some mental institutions (Healy 1976). Where prevalence of significant IHA titers is high, one may expect a high prevalence of amebic disease. Healy et al. (1970) first used frequency distribution curves for IHA titers to illustrate different levels of endemicity.

The shape of the antibody profile curve is related to the epidemiology of amebiasis in sampled populations (Healy et al. 1970, Healy and Gleason 1972, Kagan 1976). Unimodal curves skewed to the left (low titers) signify populations in which amebic disease is not endemic, although amebic infection may be prevalent, e.g., Cherokee school children (Healy et al. 1970). Bimodal curves with a second peak at titers above 1:64 signify endemic amebic disease. Bimodal or even unimodal curves skewed to the right (high titers) suggest high prevalence of invasive amebiasis. Hemagglutination titers for amebiasis were included in the U.S. Health and Nutrition Survey conducted by the National Center for Health Statistics (Healy 1976). The results from this survey should provide a better assessment of amebiasis as a public health problem in the United States.

Knight (1975) suggested serology and fecal examination for cysts as the only practical means of assessing infection rates with E. histolytica. A simple deterministic mathematical model of amebic infection was devised to estimate rates for gain and loss of infection and change in seropositivity. Well-designed epidemiological studies should be done to test the validity of this model.

Intestinal Amebiasis

An expert committee of the World Health Organization (1969) classified amebiasis into asymptomatic and symptomatic cases, the latter including intestinal and extra-intestinal amebiasis.

Classification of Intestinal Amebiasis

The two main clinical entities of intestinal amebiasis are amebic dysentery and nondysenteric amebic colitis. Amebic dysentery is due to tissue-invasive Entamoeba histolytica with clinical manifestations being attributable to the parasite leaving its commensal existence in the lumen of the colon and becoming a true parasite. Although numerous authors have attributed a wide spectrum of symptoms to this ameba without evidence of tissue invasion, Wilmot (1962) found it difficult to attribute symptoms to the ameba remote from its habitat. Neither Elsdon-Dew (1968) nor Adams and MacLeod (1977a) accept nondysenteric colitis as a clinical entity attributable to amebic infection. Ameboma is a tumor in the colon consisting of granulation tissue that contains trophozoites of E. histolytica and may occur anywhere in the colon, most commonly in the cecum (Wilmot 1962, Adams and MacLeod 1977a). Amebic appendicitis occurs in a small number of cases with extensive ulceration of the cecum (Wilmot 1962).

Further discussion of intestinal amebiasis is limited to amebic dysentery.

Clinical Manifestations

Clinical manifestations of amebic dysentery have been reviewed by Anderson et al. (1953), Juniper (1962), Wilmot (1962), and Adams and MacLeod (1977a). Human experimental infections show prepatent periods from one to 126 days (Beaver et al. 1956). In the classic study by Walker and Sellards (1913), the prepatent period in 16 prisoner volunteers was between one and 44 days with a median of 4.5 days. In the study by Beaver et al. (1956) the median prepatent period was 5 days. The prepatent period refers to the time from ingestion of amebic cysts to appearance of amebae in feces, whereas incubation period refers to the time from infection to appearance of symptoms. The incubation periods for amebic dysentery in four male prisoners at Bilibid Prison in Manila were 20, 57, 87, and 95 days (Walker and Sellards 1913). Craig (1944) reported data from an outbreak of amebic dysentery among the military in El Paso, Texas, between July and November 1916. The incubation period varied from one week to one year with a median of two months after arrival in the area. Sartwell (1950) analyzed data from the 1933 Chicago outbreak and calculated a median incubation period of 21 days.

Wilmot (1962) found that most cases of amebic dysentery have a gradual onset with a course marked by intermittent symptoms. Patients with amebic dysentery complain of dysenteric stools often preceded by diarrhea (Wilmot 1962). Rectal bleeding and bloody mucus in feces are the next most common symptoms of amebic dysentery. Abdominal pain is a frequent symptom, often accompanied by low back pain (Wilmot 1962, Adams and MacLeod 1977a). Symptoms reported less frequently are weight loss, nausea and vomiting, and flatulence (Wilmot 1962, Juniper 1962, Adams and MacLeod 1977a).

The most frequent physical sign in amebic dysentery is the presence of ulcerations in the colon viewed by sigmoidoscopy (Juniper 1962, Wilmot 1962, Adams and MacLeod 1977a). Abdominal tenderness is a frequent sign, and a significant proportion of cases have an enlarged, tender liver without abscess (Juniper 1962, Wilmot 1962, Adams and MacLeod 1977a). Fever is present in a minority of patients, often the more seriously ill (Adams and MacLeod 1977a). Abdominal distention and dehydration are seen infrequently and tend to occur in severely ill patients (Adams and MacLeod 1977a).

Complications and Sequelae of Amebic Dysentery

Complications and sequelae of amebic dysentery have been discussed by Anderson et al. (1953), Wilmot (1962), and Adams and MacLeod (1977a). Amebic liver abscess (ALA) is by far the most common complication of amebic dysentery. Peritonitis is the next most common complication and the most dangerous with a case fatality rate of 40% (Adams and MacLeod 1977a). Hemorrhage is presumed to occur from erosion of a blood vessel in the floor of an amebic ulcer; the prognosis is grave (Wilmot 1962). Intussusception is a rare complication associated with an ameboma or thickened bowel wall and may reduce spontaneously. Otherwise, laparotomy is indicated (Wilmot 1962, Adams and MacLeod 1977a). Strictures in the large bowel can be attributed to E. histolytica by identifying the ameba in biopsy specimens and by response of the lesions to treatment with tissue amebicides (Adams and MacLeod 1977a).

Diagnosis

Key points in the diagnosis of amebic dysentery were covered by Juniper (1962), Wilmot (1962), Stamm (1970), Pittman et al. (1973a), and Krogstad et al. (1978a).

Workers in Durban, South Africa, insisted on finding hematophagous trophozoites before making a diagnosis of amebic dysentery (Wilmot 1962, Adams and MacLeod 1977a).

Other investigators may (Juniper 1962) or may not insist upon this criterion (Buchan 1968, Pittman et al. 1973a). It is generally accepted that hematophagous trophozoites are tissue invasive (Elsdon-Dew 1968).

Other members of the genus Entamoeba must be differentiated from E. histolytica when they occur in the gut of man. With the recognition of E. hartmanni as a distinct species, it is no longer appropriate to refer to large and small races of E. histolytica (World Health Organization 1969). Burrows (1957, 1959, 1964) and Gleason et al. (1963) published authoritative discussions on E. hartmanni. Entamoeba polecki is another species whose taxonomy is confused (Levine 1973) and whose pathogenic potential in man is still debated (Salaki et al. 1979). E. histolytica-like amebae, which are morphologically identical to E. histolytica but can grow at room temperature, were discussed by Goldman (1969). He considered the free-living species E. moshkovskii found in sewage and water to be closely related to E. histolytica-like amebae of the Laredo type. From analysis of nucleic acids and genome size Gelderman et al. (1971a, b) suggested that classic E. histolytica, E. histolytica-like amebae, and E. moshkovskii constitute separate species. In their classic paper of 1913, Walker and Sellards proved conclusively that Entamoeba

histolytica is pathogenic whereas E. coli is not. They emphasized the importance of microscopic diagnosis in amebiasis and the differentiation of E. coli from E. histolytica to avoid indiscriminate treatment of everyone having Entamoeba species in their stools.

An accurate diagnosis is unlikely to be made without an adequate specimen being examined with the proper techniques by a competent parasitologist. Methods for detection of amebic antigen in feces by enzyme immunoassay may help alleviate some of the difficulties with microscopic diagnosis of E. histolytica (Root et al. 1978, Palacios B. et al. 1978). Sigmoidoscopy can be a valuable aid to diagnosis (Juniper 1962, Wilmot 1962, Gilman and Prathap 1971, Pittman et al. 1973a, Adams and McLeod 1977a, Krogstad et al. 1978a). Wilmot (1962) described the appearance of normal and abnormal intestinal mucosa by sigmoidoscopy. The radiological appearance of intestinal amebiasis is that of ulceration, inflammation, and fibrosis (Weinfeld 1966); but radiology has little value in the diagnosis of uncomplicated intestinal amebiasis (Wilmot 1962). Radiology can be very important in the differential diagnosis of intestinal amebiasis with complications (Tchang 1968), particularly ameboma (Pittman et al. 1973c). A variety of serologic tests is useful when the parasite is tissue invasive, but

they have little value when the ameba is commensal. Healy and Cahill (1971) moderated a panel discussion on the serology of amebiasis as a diagnostic tool, and Kagan (1976) reviewed the subject. Pathophysiological changes in amebic dysentery are nonspecific and therefore, little help in the differential diagnosis (Powell (1959a).

Pathology and Pathogenesis

Councilman and Lafleur (1891) lucidly described the pathology of amebic dysentery. For the most part, their astute observations have stood the test of time. One must remember they were working with autopsy material and had no way of controlling for postmortem changes (Anderson et al. 1953). Comprehensive reviews of the subject have been published by Anderson et al. (1953), Brandt and Pérez Tamayo (1970), and Pérez-Tamayo and Brandt (1971).

The earliest lesions of intestinal amebiasis were not recognized until Prathap and Gilman (1970) studied rectal biopsies from Orang Asli aborigines in Malaysia. The mildest lesion they described is characterized by thickening of the mucosa with irregular wavy surface. The surface epithelium is intact, yet the lamina propria is edematous and inflamed. Capillaries in the lamina propria are distended. Although the surface epithelium is intact, scattered foci of the cells are cuboidal. It may be difficult to

attribute the nonspecific lesion described by Prathap and Gilman to Entamoeba histolytica because amebae were not found in glands or in the tissue. Nevertheless, Pittman et al. (1973b) described the same lesion from rectal biopsies taken from patients with amebiasis and used similar terminology for a diffuse lesion indistinguishable from nonspecific inflammation in other kinds of colitis. A variety of ultrastructural changes occur in the surface epithelium.

Pittman et al. (1973b) described focal lesions of the intestinal mucosa corresponding to micro-ulcerations described by Prathap and Gilman (1970). In both reports, amebae were found at the surface of these ulcers but not in the tissue. The surface epithelium is flattened or in some cases completely denuded. There is inflammation of the lamina propria. Edema seems to lift the epithelium from the underlying lamina propria in which dilated blood vessels are often involved with capillary hemorrhage. Amebae are plentiful in the exudate. The ulcers do not extend below the muscularis mucosae nor do they form the typical flask-shaped ulcer at this stage. Pittman et al. (1973b) described the ultrastructural changes. Contact with amebae does not seem to damage most tissue cells. On the other hand, many amebae were in various stages of lysis. Inflammatory cells in tissue apparently have little effect on

amebae but are routinely ingested by the amebae without the loss or rupture of their cell membrane (Griffin 1972).

Prathap and Gilman (1970) had the advantage of studying material with lesions more advanced than the most severe lesions studied by Pittman et al. (1973b). The surface epithelium between glands is destroyed before amebae invade tissue. The amebae penetrate through the basement membrane and into the superficial area of the lamina propria but stop short of the muscularis mucosae. There is little inflammation or tissue necrosis at the site of invasion; but necrosis and neutrophils lie just beyond the advancing amebae, between them and normal tissue.

The typical flask-shaped ulcer of intestinal amebiasis represents a relatively late stage in the invasive process (Prathap and Gilman 1970). The earliest lesions described by Councilman and Lafleur (1891) had already reached this stage. The lesion extends through the mucosa and muscularis mucosae into the submucosa with a thick exudate containing trophozoites at the floor of the ulcer. A necrotic zone lies beneath the exudate and demarcates it from the viable submucosa in which there is a prominent inflammatory process. When the mucosa and muscularis mucosae are lost, granulation tissue forms in their place (Prathap and Gilman 1970).

As the pathological process develops, further undermining of the intestinal mucosa results in the formation of interconnecting tunnels between adjacent ulcers (Anderson et al. 1953). The amount of inflammation usually increases and is accompanied by severe tissue damage. The colon takes on a shaggy appearance due to the undermined mucosa and may eventually show complete denudation of the epithelium.

Many of the observations by Prathap and Gilman (1970) and Pittman et al. (1973b) have been confirmed in an experimental model (Takeuchi and Phillips 1975, 1976a, b; Takeuchi et al. 1977). Takeuchi and his associates used young germfree guinea pigs, which had been given the flora from human cases of symptomatic amebiasis, for cecal inoculation with Entamoeba histolytica.

Takeuchi and Phillips (1975) observed leukotaxis of polymorphonuclear leukocytes at the site of amebic invasion. The relative absence of PMN in amebic lesions of the intestine (Councilman and Lafleur 1891) and in stools of amebic dysentery (Beaver 1959, Stamm 1970) is probably due to the disintegration of large numbers of these cells (Takeuchi et al. 1977). Takeuchi et al. (1977) postulated that disintegrating PMN may be responsible for the histolytic action seen in the gut mucosa with amebic dysentery.

Treatment

A detailed discussion on the chemotherapy of amebiasis is beyond the scope of this work. Current recommendations for treatment are given in the Medical Letter on Drugs and Therapeutics (Abramowicz 1982). Emetine, one of the first drugs used to treat amebiasis, is still part of the pharmacopia. Wilmot (1962) gave a thorough discussion on the treatment of this disease in his book on clinical amebiasis. For a scholarly review of the subject, consult Steck (1971). The subject was also reviewed by Woolfe (1965), Juniper (1966), Powell (1969, 1971, 1972), Thompson (1970), and Knight (1980). Krogstad et al. (1978a) included treatment in their overview of amebiasis.

Amebic Hepatitis

Rogers (1922) recognized two forms of amebic liver disease, amebic liver abscess and "presuppurative amoebic hepatitis." He classified these cases as amebic hepatitis because the patients present with fever and leukocytosis without signs of liver abscess and respond dramatically to treatment with ipecacuanha. He concluded that amebae reach the liver via the portal vein and cause amebic hepatitis whether or not an abscess eventually forms. Ochsner and DeBakey (1943) agreed that amebae must enter the liver and cause hepatitis before the formation of abscesses. Clinical

manifestations of amebic hepatitis include fever, pain and tenderness over the liver, hepatomegaly, and moderate leukocytosis. Because the diagnosis of amebic hepatitis is based largely upon clinical impression, reliable estimates of prevalence are lacking.

Workers in Greece (Doxiades et al. 1961, Doxiadis and Candreviotis 1961, Doxiades 1962, Doxiades et al. 1963, Doxiadis and Moschoutis 1970) waged a campaign in the literature to recognize amebic hepatitis as a clinicopathological entity. The evidence from Greece is unconvincing, however, because published photomicrographs of putative amebae (Doxiades et al. 1961, Doxiadis and Candreviotis 1961, Doxiades 1962) do not show diagnostic morphological features. Amebic hepatitis was supposedly produced in a guinea pig by injection of ascitic fluid from a patient with amebic hepatitis (Doxiades et al. 1961), but bona fide amebae are not evident in the published photomicrograph. In the same paper, much emphasis was placed on the therapeutic response of patients with amebic hepatitis to treatment with antiamebic drugs; yet amebae were said to be found in a liver biopsy 16 days after a course of emetine and chloroquine. Radiography and liver scans are used to help make the diagnosis (Doxiades and Stergiou 1964, Doxiadis and Moschoutis 1970). Besides the failure to

demonstrate amebae unequivocally in cases of amebic hepatitis, Doxiadis and associates always neglected to do serologic tests for antibody against Entamoeba histolytica.

Other workers have continued to use the term and investigate the pathological basis for a clinical picture called amebic hepatitis (Kasliwal et al. 1967, Tandon and Rajan 1968, Reddy et al. 1969, Tandon et al. 1975). Powell et al. (1959) disliked the term amebic hepatitis and considered the hepatomegaly and tenderness associated with amebiasis to be due to a nonspecific hepatitis caused by intestinal ulceration.

The subject may not yet be closed because Mahajan et al. (1976) detected amebic antigen in two liver biopsy specimens from patients with amebic hepatitis. Serology for amebiasis was reactive by counterimmunoelectrophoresis, but the evidence would have been more convincing if they had demonstrated bands of identity by double diffusion in agar gel.

Rees et al. (1954) provided the only experimental evidence for amebic hepatitis by culturing amebae from the livers of guinea pigs that had been inoculated intracecally with E. histolytica. None of the livers showed

histopathological evidence of amebae or damage attributable to them.

Immunology of Amebiasis

Entamoeba histolytica causes an immune response in man and experimental animals. This review of the literature partially describes that immune response but does little to explain the immunologic determinants of this disease. If the immune system helps determine whether an individual develops amebic disease or merely harbors a commensal intestinal ameba, then those immunologic factors that predispose to amebic disease are unknown.

Innate Resistance

Balamuth and Siddiqui (1970) and Kagan (1973) stated that solid evidence for innate resistance to *E. histolytica* in man is lacking although few people who harbor the ameba develop disease from it. In animals the principal evidence for innate resistance comes from guinea pigs, which are highly susceptible to cecal infection but not direct infection of the liver unless the animal has seen amebic antigen or amebae in the gut (Maegraith and Harinasuta 1959).

Acquired Immunity

Common reinfection with intestinal amebiasis is considered evidence against acquired immunity to amebiasis in man (Balamuth and Siddiqui 1970), but the same argument may not apply to amebic liver abscess. Rogers (1922) was convinced from studying autopsy material in Calcutta that ALA sometimes results in "dying out of the amoebic infection." Sepúlveda (1976) and Krupp and Jung (1976) said that recurrence of ALA following successful treatment is rare. Vázquez-Saavedra et al. (1973) noted that some hamsters resist liver infection after initial hepatic amebiasis is cured by chemotherapy. Thus, a weak case can be made for acquired immunity to amebic liver abscess in animals and man.

Spontaneous cure of intestinal amebiasis in dogs was documented by Faust (1932). Swartzwelder and Avant (1952) found 24 of 29 dogs refractory to reinfection with the same strain of ameba or a heterologous one following termination of an initial experimental infection of the gut by spontaneous cure or chemotherapy. Jain et al. (1980) immunized young guinea pigs by inoculating live axenic amebae into the mesenteric vein or by inoculating guinea pigs intracecally with monoxenic amebae followed by treatment with metronidazole. The animals were partially

protected against intracecal challenge with pathogenic E. histolytica. Definitive experiments on acquired immunity after amebic infection are lacking.

Humoral Immunity

A protective function for humoral immunity in amebiasis has not been conclusively demonstrated. Maddison (1965) and Krupp (1970) stated that antibodies against E. histolytica are not protective in man. Sepúlveda (1980a) disagreed and presented evidence to the contrary for amebic liver abscess.

Passive transfer of immunity has been attempted in dogs and hamsters. Swartzwelder and Avant (1952) transferred blood from dogs that were refractory to reinfection with E. histolytica into recipient animals. Seven of 10 transfused dogs resisted infection per anum while 85% of the control animals were susceptible to infection. Of course, this crude experiment did not necessarily suggest a protective function for antibody because cells and other blood constituents were transferred with serum. When human immune serum is inoculated intraperitoneally into hamsters before intrahepatic challenge with pathogenic amebae, the hamsters are partially protected in terms of less extensive lesions compared to control animals (Sepúlveda et al. 1974).

The humoral response in amebiasis is characterized by elevated levels of serum IgG in cases of symptomatic

intestinal amebiasis (Abioye et al. 1972, Dasgupta 1974) and amebic liver abscess (Perches et al. 1970, Abioye et al. 1972). Immunoglobulin A may also be increased in sera from ALA patients (Perches et al. 1970). Amebiasis patients usually have IgM levels comparable to those in healthy individuals or cyst passers (Abioye et al. 1972, Dasgupta 1974). Dasgupta (1974) reported raised serum concentrations of IgE in amebiasis patients without other intestinal parasites. Serologic reactions specific for antibodies to E. histolytica are attributed to IgG (Maddison et al. 1968b, Lee et al. 1970) although IgM is involved in hemagglutination (Capín-Gutiérrez et al. 1973). Using immunofluorescence, Osisanya and Warhurst (1980) found disappointingly low titers for IgM and IgA in two and three out of five sera, respectively, from ALA patients who had reasonably high titers of specific IgG. Similar results obtained with sera from patients with invasive intestinal amebiasis. Surprisingly little work of this nature has been done despite the reliable tools of immunofluorescence and enzyme immunoassay. Kagan (1973) urged investigation of secretory IgA in intestinal amebiasis. Complement fixing and hemagglutinating antibodies to amebic antigen may be detected in feces of patients with intestinal amebiasis (Shaalan and Baker 1970, Mahajan et al. 1972a).

Over the years a wide variety of serologic tests has been used to aid in the diagnosis of amebiasis (table 53). Complement fixation was the first (Craig 1927, 1929) but has been supplanted by other tests in most laboratories. Axenic culture of E. histolytica (Diamond 1961, 1968b) and methods for preparing antigen (Thompson et al. 1968) heralded a proliferation of work in this area. A few tests not listed in the table have been developed for amebiasis without benefit of thorough clinical evaluation, i.e., radioimmunoassay (Ben-Efraim et al. 1969, Voller et al. 1977), immunohistoperoxidase (Durosoir et al. 1974), surface fixation test (Ruiz Castañeda 1976), and surface coagglutination of protein A staphylococci on amebae (Culbertson and Harper 1980). Kagan (1980a, b) has written excellent chapters on technical aspects of serodiagnosis including tests for amebiasis. Today the technology exists to assay serum for amebic antigen and different immunoglobulin classes specific for E. histolytica. A new generation of highly sensitive and specific immunoassays will soon be applied to the diagnosis of amebiasis in individuals and populations.

In experienced hands with proper attention to quality control, several tests listed in table 53 give satisfactory results. In the United States indirect hemagglutination

(IHA) has long been the test of choice, but the Centers for Disease Control found it to be less useful in early amebic disease (see Stevens et al. 1979) and have replaced it with FIAX, a fluorescent immunoassay read by fluorometer (Taylor and Perez 1978) (Dr. Irving G. Kagan, personal communication).

The following recommendations consider sensitivity and specificity of a test, its relative complexity, availability of reagents and equipment, and cost. For diagnostic purposes in the field or in developing nations, one should consider double diffusion in agar gel, latex agglutination, and possibly counterimmunoelectrophoresis or enzyme immunoassay read visually. In addition to any of these, indirect hemagglutination and indirect immunofluorescence are suitable for many small laboratories in the United States or overseas. Two other tests, FIAX and spectrophotometric EIA, are available to more sophisticated laboratories with the proper equipment including computers. None of the serologic tests for amebiasis is currently useful in diagnosing asymptomatic cyst passers.

TABLE 53

SEROLOGIC TESTS FOR AMEBIASIS

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
BPH	X/K9*	NA**	96(24)***	95(22)***	100(35)#	184,185##
CF	X	NA	63(363)	97(29)	100(58)	139
	M/DKB	28(101)***	90(92)	100(20)	100(101)	254
	X	NA	16(45)	100(5)	100(10)	244
	X	NA	47(126)	100(23)	97(211)	251
	A/200:NIH,HK9	11(19)	63(30)	84(37)	100(10)	532
	M	NA	82(11)	85(27)	86(51)	7
	A/200	6(18)	52(543)	74(745)	84(128)	277
	A/NIH:200,HK9	58(33)	76(88)	88(16)	NA	231
	A/HK-9	77(22)	NA	NA	97(98)	468
	A	NA	76(17)	86(63)	52(25)	331
EIA	A	50(26)	63(8)	100(2)	91(45)	50
	A	NA	NA	95(21)	95(21)	547
	M	NA	NA	100(37)	100(20)	146
	A/HK9	0(14)	NA	92(12)	98(340)	499
	A/NIH200,HK9	14(50)	100(8)	100(12)	94(69)	290
IMMOB	live amebae	NA	90(10)	88(48)	82(82)	38

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
IMMOB	live amebae	NA	87(30)	91(60)	90(371)	36
	live amebae	40(10)	NA	100(11)	76(17)	83
	live amebae	NA	100(3)	89(19)	100(27)	425
	live amebae	NA	82(11)	73(27)	80(51)	7
Immunofluorescence tests						
FIAX	A/HK-9	43(7)	90(20)	100(33)	98(217)	525
IIF	X/K9	55(20)	84(32)	91(23)	65(17)	173
	X	92(13)	75(32)	91(33)	100(94)	44
	X	33(6)	80(10)	100(18)	79(95)	87
	X, HL	18(17)	91(23)	100(42)	100(208)	12
	X/YS9	50(10)	NA	100(11)	94(17)	83
	NA	0(37)	75(40)	95(61)	100(514)	223
	M/ABRM	NA	100(4)	84(19)	67(3)	329
	M	NA	100(11)	96(27)	100(51)	7
	HL/HK-9	NA	NA	90(42)	89(27)	182
	A/HK9	NA	NA	89(46)	90(50)	330
	X	15(20)	59(103)	100(330)	100(1217)	13
	X	NA	93(27)	99(73)	97(177)	69

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
IIF	X	NA	35(54)	100(28)	98(125)	154
	M	NA	92(12)	95(19)	100(12)	323
	M	60(15)	100(21)	100(47)	92(52)	380
	X	NA	51(76)	100(45)	98(220)	431
	NA	NA	80(5)	100(9)	91(22)	503
	M	44(9)	70(20)	73(15)	100(44)	442
	M	NA	88(43)	92(51)	96(50)	327
	A, X	30(109)	54(142)	100(412)	100(2000)	11
	NA	NA	100(16)	100(40)	NA	535
	A/200NIH	9(44)	73(40)	96(25)	96(111)	528
SAFA	A	100(8)	100(12)	100(15)	93(251)	176
Particle agglutination tests						
BF	M/DKB	0(24)	86(50)	93(90)	100(279)	538
	M	NA	86(43)	92(51)	94(50)	327
	M	45(29)	95(20)	88(26)	98(43)	542
IHA	M/DKB	66(140)	98(133)	100(35)	97(101)	254
	M	42(24)	92(66)	98(65)	83(275)	316
	M/DKB	9(70)	82(83)	97(121)	99(994)	355

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
IHA	X/K9	NA	85(20)	86(21)	NA	185
	M/DKB	NA	100(19)	100(3)	NA	345
	M/DKB	0(7)	85(63)	NA	99(98)	193
	M/DKB	NA	100(13)	100(24)	NA	312
	A/200:NIH,HK9	58(19)	90(41)	100(48)	83(12)	532
	X	64(11)	63(8)	93(14)	67(45)	437
	M/HB301	NA	NA	91(47)	84(342)	467
	A/200:NIH,HK9	16(56)	100(11)	100(6)	NA	263
	A/NIH200	9(75)	81(168)	87(31)	93(118)	272
	X	NA	57(23)	86(22)	82(51)	424
	A/HK9	NA	71(14)	93(15)	90(10)	424
	A	5(21)	85(161)###	NA	99(175)	476
	M	NA	91(11)	93(27)	94(51)	7
	A/200	44(18)	92(543)	95(745)	62(128)	277
	A	NA	NA	89(38)	NA	483
	M/DKB	NA	NA	NA	99(658)	200
	A/NIH:200,HK9	58(36)	88(88)	88(16)	NA	231
	M	NA	83(12)	95(19)	83(12)	323
	NA	NA	80(5)	89(9)	100(22)	503
	A/200	NA	100(10)	100(91)	100(42)	273

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
IHA	M	NA	93(43)	94(51)	92(50)	327
	M	11(9)	65(20)	67(15)	91(46)	442
	A/HK-9	78(23)	NA	NA	94(138)	468
	M	NA	88(17)	90(57)	84(166)	543
	X	NA	NA	90(40)	90(60)	324
	A	NA	82(17)	83(63)	88(25)	331
	A	NA	100(3)	96(25)	100(74)	381
LA	A	NA	96(100)	98(100)	93(300)	366
	A	0(8)	65(34)	93(14)	91(108)	35
	A	NA	88(17)	85(13)	81(36)	398
	A	NA	61(54)	82(28)	86(125)	154
	A	NA	75(12)	84(19)	83(12)	323
	A/HK9,200	NA	38(26)	100(11)	91(200)	364
	A	NA	100(10)	NA	100(3)	369
	A	27(15)	43(12)	89(47)	90(52)	380
	A	NA	80(15)	90(88)	93(103)	455
	A	8(50)	95(38)	97(62)	85(150)	202
	A	NA	53(76)	87(45)	90(220)	431
	A	NA	60(5)	89(9)	100(22)	503

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
LA	A	NA	100(1)	100(3)	97(153)	446
	A/HK-9	26(23)	NA	NA	NA	468
	A	NA	88(16)	97(62)	44(25)	331
PCA	M/DKB	NA	77(13)	77(24)	99(78)	312
Precipitin tests						
CAP	A	NA	100(124)###	NA	100(66)	504
	A/200NIH	NA	80(5)	80(5)	NA	392
	NA	NA	88(16)	100(40)	NA	535
CIEP	A	NA	NA	98(50)	94(52)	483
	A	NA	82(76)	93(45)	94(220)	431
	A/200	NA	96(74)	100(27)	100(42)	273
	X	NA	NA	95(40)	98(60)	324
	A	NA	82(17)	85(104)	64(25)	331
	A/HK9,NIH200	NA	69(16)	100(40)	100(100)	535
CTP	A, M	NA	89(150)	97(150)	95(240)	415
	M/DKB	NA	30(10)	35(20)	100(10)	455

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
DD	M/O	37(57)	89(164)	97(85)	93(592)	311
	M	50(24)	91(66)	97(65)	83(275)	316
	M	40(51)	88(231)	97(151)	94(1824)	315
	M	NA	NA	96(360)	88(373)	422
	X/NIH-200	52(33)	85(60)	NA	NA	19
	M	NA	92(400)	NA	82(497)	421
	M	62(13)	66(32)	94(33)	100(22)	44
	X/K9	NA	95(22)	94(16)	NA	185
	M/DKB	NA	63(19)	67(3)	NA	345
	M/N	NA	100(13)	100(24)	79(78)	312
	M	NA	96(150)	99(150)	86(240)	415
	A/200:NIH,HK9	0(19)	54(41)	80(49)	100(12)	532
	M/HB301	0(2)	67(6)	97(93)	98(443)	467
	A/200:NIH,HK9	2(51)	82(11)	100(6)	NA	263
	A	0(21)	60(161)###	NA	100(175)	476
	M,A/200	39(18)	92(543)	97(745)	88(128)	277
	A/NIH:200,HK9	52(27)	76(89)	88(16)	NA	231
	A/HK9	NA	50(26)	100(11)	100(200)	364
	M/DKB	NA	30(10)	45(20)	100(10)	455
	NA	NA	40(5)	89(9)	91(22)	503

TABLE 53--Continued

Test	Antigen	Sensitivity: Intestinal Amebiasis, Asymptomatic	Sensitivity: Intestinal Amebiasis, Symptomatic	Sensitivity: Extra-intestinal Amebiasis	Specificity	Source
DD	A/200	NA	86(74)	100(27)	100(42)	273
	A/HK9	NA	100(3)	92(25)	100(74)	381
IEP	M/"B301	0(2)	67(6)	97(93)	98(443)	467
	A/200	NA	100(448)###	NA	NA	278
	A	NA	83(23)	97(71)	NA	69
	M	67(3)	100(12)	100(29)	75(4)	380
	A/HK9	NA	NA	93(56)	NA	466
	A/HK9	65(23)	NA	NA	NA	468
TIA	A	NA	100(3)	100(25)	96(74)	381

* Source of antigen/strain of Entamoeba histolytica: A = axenic, M = monoxenic, X = xenic, HL = infected hamster liver.

** NA = information not available.

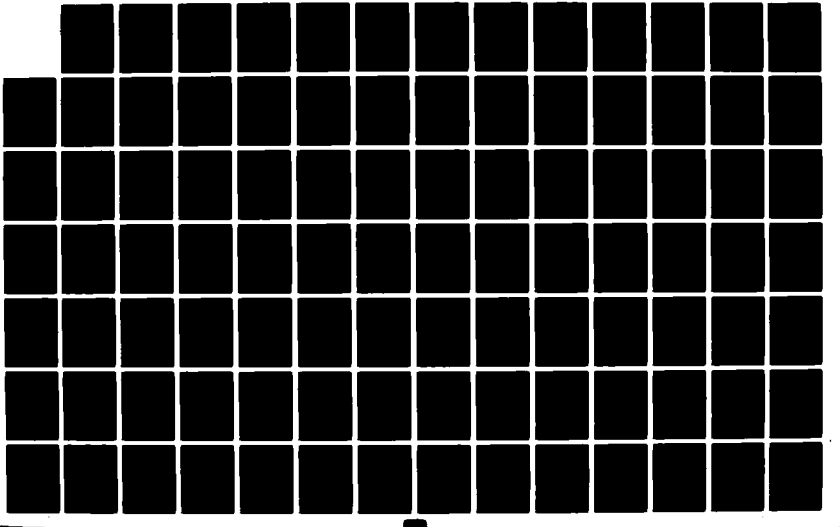
*** Percentage of true positives, number of sera in parentheses.

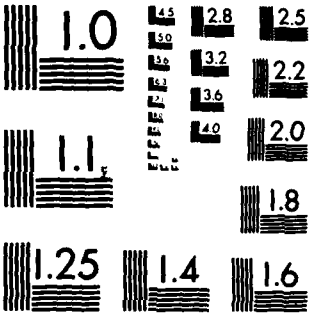
\# Percentage of true negatives, number of sera in parentheses.

Number of reference in Literature Cited.

Invasive amebiasis without distinction between amebic dysentery and extra-intestinal amebiasis.

AD-A128 689 AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY 5/6
TO ENTAMOEBA HISTOLYTICA(U) JOHNS HOPKINS UNIV
BALTIMORE MD R G TAYLOR 15 APR 83 DAMD17-75-C-5001
UNCLASSIFIED F/G 6/5 NL





ABBREVIATIONS FOR TESTS:

BF	Bentonite flocculation
BPH	Bentonite phagocytosis
CAP	Cellulose acetate membrane precipitin
CF	Complement fixation
CIEP	Counterimmunoelectrophoresis
CTP	Capillary tube precipitin
DD	Double diffusion
EIA	Enzyme immunoassay
FIAX	Fluorescent immunoassay
IEP	Immunoelectrophoresis
IHA	Indirect hemagglutination
IIF	Indirect immunofluorescence
IMMOB	Immobilization
LA	Latex agglutination
PCA	Passive cutaneous anaphylaxis (in guinea pig)
SAFA	Soluble antigen fluorescent antibody
TIA	Thin layer immunoassay

Serologic tests have two principal uses, clinical diagnosis and epidemiology. Antibody frequently persists a long time after apparent cure without reinfection (Krupp and Powell 1971a, b; Juniper et al. 1972; Ambroise-Thomas and Truong 1972; Healy et al. 1974). Healy et al. (1974) cited one example of reactive tests (IHA, CIEP, IEP) five years after treatment for intestinal amebiasis. This problem prohibits use of serologic tests for routine diagnosis of amebiasis without other evidence of infection. Krupp and Powell (1971b) attempted to differentiate between acute infections and old ones by studying the patterns of arcs in immunoelectrophoresis and combining this information with IHA titer, but the method has not gained wide acceptance.

Persistence of antibody does not pose the problem in seroepidemiology that it does in clinical application. Healy and Gleason (1972) and Kagan (1976) reviewed seroepidemiology of amebiasis at international meetings in Mexico City. Double diffusion in agar was the first serologic test for amebiasis used for epidemiological studies (Maddison et al. 1965a). Indirect hemagglutination has proved useful for this purpose in many parts of the world (Cuadrado and Kagan 1967, Cuadrado et al. 1967, Healy et al. 1970, Healy 1976, Mathews et al. 1980). Intradermal tests (Miller et al. 1973), counterimmunoelectrophoresis

(Gutiérrez et al. 1976), and enzyme immunoassay (Bos et al. 1980) have also been used successfully.

Serologic tests for amebiasis have also been used in animals. Vinayak et al. (1979b) determined IHA titers in naturally infected rhesus macaques. Bos (1973) studied the humoral response of hamsters with ALA by indirect immunofluorescence (IIF) and immunoelectrophoresis (IEP). Others have used IHA, CIEP, or EIA for this purpose (Ríos et al. 1978, Hartman et al. 1980, Norman et al. 1979, Capín et al. 1980a). Ghadirian and Meerovitch (1978b) used IHA, but Ríos et al. (1978) used CIEP to assess the immunogenicitity of amebic vaccines in hamsters. IHA, IEP, and CIEP have been used for the same purpose in guinea pigs (Krupp 1974b, Vinayak et al. 1980).

The in vitro effects of serum on Entamoeba histolytica have been studied since Dale and Dobell (1917) observed "paralysis of the amoebae" and their eventual death in the presence of serum from an infected kitten and from an uninfected dog and cat. Cole and Kent (1953) were apparently unaware of these observations when they reported immobilization of amebae by rabbit antiserum to E. histolytica and by sera from some patients with amebiasis. Biagi-F. and Buentello (1961) designed a diagnostic test for amebiasis based on the phenomenon of immobilization.

Further experiments showed that immobilization is due to IgG (Yap et al. 1969), and trophozoites regain motility after 105 minutes (Biagi F. et al. 1966).

Other in vitro effects of immune serum on amebae have been described. Rabbit antiserum to E. histolytica partially inhibits phagocytosis of erythrocytes (Shaffer and Ansfield 1956). Human immune serum inhibits in vitro growth of amebae (de la Torre et al. 1973) and their pathogenicity to hamsters after exposure to the serum in culture (Sepúlveda et al. 1974). Immune lysis of trophozoites by unheated sera in vitro (Biagi-F. and Buentello, 1961, Sepúlveda et al. 1973a) is mediated by complement and antibody (Guerrero-Alcázar et al. 1972, Sepúlveda et al. 1974, Huldt et al. 1979). In the absence of antibody, E. histolytica activates complement by the alternative pathway and the reaction products lyse them (Ortiz-Ortiz et al. 1978, Huldt et al. 1979). These findings possibly explain why nonimmune sera from the dog or cat killed amebae in the experiments by Dale and Dobell (1917).

Certain observations concerning the immobilization reaction formed a background for inquiry into the dynamics of the E. histolytica cell membrane, which may be related to survival of the parasite in its host and possibly pathogenesis of amebiasis. Biagi F. et al. (1966) originally

noted that immobilized amebae regain their motility while somehow moving fluorescent-labeled antibodies from their surface into cytoplasmic vacuoles. Under different experimental conditions, cell capping of antibody was observed (Aust-Kettis and Sundqvist 1976, 1978; Calderón et al. 1980). Antibodies bound to antigenic determinants on the surface of E. histolytica become redistributed within the cell membrane and by endocytosis within the cytoplasm (Aust-Kettis and Sundqvist 1978, 1980). Some of the antibodies rapidly dissociate from antigenic determinants although others are lost more slowly from the ameba surface as antigen-antibody complexes. Some cell-associated antibodies are internalized and partially degraded (Aust-Kettis et al. 1981). Amebae in axenic culture are also able to vary spontaneously their antigenicity in a cyclic fashion related to the growth cycle (Aust-Kettis and Sundqvist 1978). The relative abundance of surface antigens is minimal during the logarithmic growth phase but maximal during the stationary phase of peak population density. Both antigenic variation and a mechanism for disarming surface-bound antibodies would have survival value for amebae in vivo (Aust-Kettis and Sundqvist 1980, Aust-Kettis et al. 1981).

Cell-Mediated Immunity

The role of cell-mediated immunity in amebiasis has been explored primarily through intradermal tests and in vitro correlates of CMI. Interpretation of results from intradermal tests has been hampered by failing to distinguish clearly among the types of immunological reactions that may be involved. Furthermore, few authors have bothered to examine histological sections at the site of the skin reaction (Kagan 1973).

Preparation of axenic HK-9 amebic antigen (histolyticin) for skin tests (Thompson et al. 1968) and assay of its potency in the guinea pig (Lunde et al. 1969) stimulated a number of studies with human patients (table 54). Investigators had had modest success with monoxenic skin test antigens for diagnosis in South Africa (Maddison et al. 1968a) and Canada (Miller and Scott 1970). Kirkpatrick et al. (1972) found that an 8 µg dose of histolyticin is excessive for routine use because it sensitizes normal individuals. Specificity was lacking for the immediate skin reaction to 11 µg histolyticin applied to patients with ALA and amebic dysentery (Kretschmer et al. 1972) and did not improve when the dose was reduced to 4 µg (Kretschmer and López-Osuna 1971). Previous experience with *E. histolytica* could not be ruled out for normal controls

TABLE 54

INTRADERMAL TESTS FOR AMEBIASIS

Reaction	Antigen	Sensitivity			Specificity	Source
		Intestinal		Extra-Intestinal		
		Asymptomatic	Symptomatic			
Immediate*	M/DKB,1.6&0.8 µg**	NA***	69(13)#	75(24)#	94(78)##	312###
	M/LLB,1.5-3.5 µg	NA	91(23)	80(21)	100(26)	360
	A/HK-9,11&22 µg	NA	76(17)	78(51)	36(50)	267
	A/HK9,0.32 µg	82(57)#	NA	90(88)	96(48)	466
	A/HK9	39(23)	NA	NA	NA	468
Delayed@	M/DKB,1.6&0.8 µg	NA	31(13)	25(24)	90(78)	312
	M/LLB,1.5-3.5 µg	NA	70(23)	60(21)	100(26)	360
	A/HK-9,4 µg	NA	NA	62(24)	90(10)	266
	A/HK-9,11&22 µg	NA	47(17)	60(51)	80(50)	267
	A/HK-9,5 µg	NA	NA	8(13)	NA	389
	A/HK-9	17(23)	NA	NA	NA	468
	A/HK-9,5 µg	NA	NA	8(13)	84(16)	391

* Read 5-20 min after injection of antigen.

** Source of antigen/strain of Entamoeba histolytica, dose of antigen as protein: A = axenic, M = monoxenic.

*** Information not available.

\# Percentage of true positives, number of patients in parentheses.

Percentage of true negatives, number of patients in parentheses.

Number of reference in Literature Cited.

@ Read 10-12 h or 24-72 h after injection of antigen.

(Ortiz-Ortiz et al. 1975). Specificity was considerably better for delayed skin reactions but sensitivity was poor. Chromatographic fractions of the antigen offered little improvement (Kretschmer and López-Osuna 1971). Kretschmer et al. (1972) did show that predominantly mononuclear cells infiltrate the site of a delayed skin reaction to histolyticin; but thrombosis, hemorrhage, and significant edema are absent. Landa et al. (1976) described the histological appearance of a 48-hour intradermal reaction to histolyticin as similar to that elicited by purified protein derivative (PPD) of Mycobacterium tuberculosis. Therefore, amebiasis patients may exhibit delayed hypersensitivity (DH) as a positive response to skin test with amebic antigen.

Unfortunately, the picture is more complex than that. Ortiz-Ortiz et al. (1973b) observed that patients with ALA frequently lack delayed hypersensitivity to amebic antigen administered intradermally. Following antiamebic treatment, these patients all exhibit skin reactions to streptokinase-streptodornase and produce humoral antibodies to E. histolytica. Landa et al. (1976) studied the puzzling anergic state in ALA postulated by Ortiz-Ortiz et al. (1975) by assessing the immunologic status of ALA patients before and after treatment for amebiasis. Most ALA patients had DH to streptokinase-streptodornase and PPD, a normal number of T

cell rosettes, and antibodies to E. histolytica. However, delayed hypersensitivity to histolyticin was suppressed before treatment in 21 of 25 patients but was demonstrated 30 days following clinical recovery in 16 of the 25. Landa et al. (1976) interpreted these findings as transitory anergy apparently unrelated to any deficiency in T cells. The anergic state subsided as the patients improved clinically. Although these studies do not explain the anergic state accompanying ALA (if it exists), they do emphasize the need for research on the immunology of amebic liver disease.

Guinea pigs with amebic liver lesions do not respond to amebic antigen given intradermally (i.d.) even after the lesions begin to disappear (Bray and Harris 1977a). However, when a dose of live axenic amebae insufficient to produce liver lesions is inoculated into the mesenteric vein of guinea pigs, the animals develop a delayed skin reaction to amebic antigen (Jain et al. 1980). (Skin biopsies were not done.) Because the guinea pig is a poor model for amebic liver abscess, similar experiments and more definitive ones using in vitro correlates of CMI in the hamster may help answer the question about anergy in ALA.

In vitro correlates of cell-mediated immunity should give some insight to the nature of immune mechanisms in

amebiasis. Peripheral blood lymphocytes from ALA patients undergo blast transformation when exposed to amebic antigen in vitro (Savanat et al. 1973b, Harris and Bray 1976, Segovia et al. 1980), but lymphocytes from patients with invasive intestinal amebiasis do not (Harris and Bray 1976). A strong humoral response to E. histolytica and a non-specific blastogenic response to phytohemagglutinin (PHA) were intact in both groups of patients (Savanat et al. 1973b, Harris and Bray 1976). These findings led Harris and Bray (1976) to postulate specific cellular immunodepression during amebic invasion of the bowel followed by sensitization of circulating lymphocytes when amebae reach the liver. This hypothesis is consistent with finding that peripheral lymphocytes obtained from ALA patients after successful chemotherapy kill pathogenic trophozoites of E. histolytica in vitro (Guerrero et al. 1976). Lymphocytes from patients with acute ALA not only fail to kill trophozoites but are themselves killed and ingested by amebae.

Few studies on lymphocyte transformation have been done in experimentally infected animals. Bray and Harris (1977a) found that a specific blastogenic response to amebic antigen begins to wane with the spontaneous disappearance of amebic liver lesions in guinea pigs. Gold et al. (1978) attempted to study the immunology of hepatic amebiasis in

hamsters, but difficulties with axenic cultures and an unexpected decrease in pathogenicity of the amebae complicated interpretation of their results. Blastogenic response of spleen cells from infected hamsters was generally low to amebic antigen. The same was true for stimulation of spleen cells by PHA and ConA; therefore, immunosuppression in the hamsters may have been generalized (Gold et al. 1978).

A more elegant in vitro correlate of CMI than lymphocyte transformation is the lymphokine macrophage inhibitory factor (MIF). At the time of admission to hospital, peripheral lymphocytes from most ALA patients did not produce MIF in culture (Ortiz-Ortiz et al. 1975). Ten days after successful antiamebic treatment all the patients had CMI to E. histolytica evident by MIF production. Delayed hypersensitivity to histolyticin was confirmed by skin biopsy and showed parallel development with MIF production. These findings are similar to an observation by Savanat et al. (1973b), who demonstrated development of a blastogenic response to amebic antigen by lymphocytes from an ALA patient during the course of his illness. Ganguly et al. (1979) reported MIF production for patients with amebic liver abscess and intestinal amebiasis, but not enough details were given to ascertain whether adequate controls were included in the assay.

MIF production has also been studied in experimental animals. Jain et al. (1980) provided few details about the assay and sketchy data before reaching the wrong conclusion about their experiment; they interpreted migration inhibition of macrophages as an indication of depressed CMI. Actually, in their experiment guinea pigs with amebic ulcers of the cecum displayed some degree of cellular immunity to E. histolytica. After guinea pigs were inoculated intrahepatically with xenic amebae, migration of macrophages from the same animals was assayed by incubating peritoneal exudate cells (containing lymphocytes and macrophages) with amebic antigen (Bray and Harris 1977a). The direct MIF assay is negative at first (four days) but becomes strongly positive eight days after infection. Strong MIF production is correlated with resolution of liver lesions and is waning when amebic lesions are healed at 12 days. Therefore, at least one measure of cell-mediated immunity, i.e., MIF, in the guinea pig does not correlate with the analogous situation in man.

In hamsters with ALA, production of MIF is associated with the absence of detectable liver lesions early in the infection (five days) and the disappearance of lesions 25 days or more after infection (Ortiz-Ortiz et al. 1973a). Between 10 and 20 days post infection when lesions are

demonstrable, direct assay for MIF is negative. Gold et al. (1978) assayed for MIF production by hamster lymphocytes using peritoneal macrophages from guinea pigs and found a similar alternating pattern of MIF activity. The absence of MIF approximately two weeks after experimental hepatic infection in hamsters may correspond to acute ALA in man when CMI is depressed. After chemotherapy, CMI is evident by MIF from human lymphocytes. Perhaps MIF production in hamsters three weeks after infection represents an analogous phenomenon.

Diamantstein et al. (1980) showed that mouse spleen cells depleted of T lymphocytes have a greatly diminished in vitro response to extracts of E. histolytica and ConA, a T cell mitogen, but a normal response to LPS, a B cell mitogen. Conversely, mouse spleen cells depleted of B lymphocytes have a slightly diminished response to E. histolytica, normal response to ConA, and greatly reduced response to LPS. Also, spleen cells from athymic nu/nu mice (deficient in functional T cells) have a significantly reduced response to amebic extract but nevertheless respond weakly to amebic mitogen, unlike ConA. These data indicate that E. histolytica may interact predominantly with T lymphocytes from the mouse but not necessarily to the exclusion of B cells

(Diamantstein et al. 1980). Whether these findings pertain to man or animal models for amebiasis remains to be seen.

Polymorphonuclear leukocytes may be involved in the pathogenesis or immunology of amebiasis. Jarumilinta and Kradolfer (1964) observed that live E. histolytica trophozoites have a toxic effect on PMN in vitro. Granulocytes from a wide variety of animals including man are susceptible. When heat damaged amebae are presented to PMN, the leukocytes engulf them. Other authors confirmed these observations (Artigas et al. 1966, Chévez et al. 1972, Chévez and Segura 1974). Toxic killing of leukocytes is a property of increased pathogenicity in axenically cultured E. histolytica (Bos and van de Griend 1977). Peripheral PMN in whole blood from patients with amebic dysentery or ALA phagocytize bentonite particles coated with amebic antigen (Halpern et al. 1967), but PMN from normal individuals or patients with other diseases do not (Halpern et al. 1962). Phagocytic function of peripheral PMN from ALA patients seems depressed when granulocytes are presented heat-killed bacteria in vitro (Ghosh and Sen 1980). In the gut wall of experimentally infected guinea pigs, E. histolytica is selectively toxic to PMN (Takeuchi and Phillips 1976a, b), which are attracted to the site of tissue invasion (Takeuchi and Phillips 1975). Entamoeba histolytica possesses a

cytotoxin-enterotoxin (Lushbaugh et al. 1978a, 1979) that is immunogenic in man and, therefore, naturally occurring in human amebic infection (Lushbaugh et al. 1980a). Takeuchi et al. (1977) postulated that histolysis of the mucosa in amebic dysentery may be due to disintegrating PMN.

The macrophage is another inflammatory cell that is likely involved in the immunology of amebiasis. Bos and van de Griend (1977) found that axenic amebae are toxic to macrophages from guinea pigs. Entamoeba histolytica liberates heat labile substance(s) into axenic culture medium that inhibits chemotaxis by human monocytes in Boyden chambers (Kretschmer 1980, Kretschmer and Collado 1980). Peripheral monocytes from patients with ALA or amebic dysentery have normal phagocytic function (Ghosh and Sen 1980). In contrast, hamsters with hepatic amebiasis have less effective phagocytic function of the reticulo-endothelial system (Capín et al. 1980a). The possible role of macrophages in the immunology of amebiasis is barely explored, although Ghadirian and Meerovitch (1982) found that activated macrophages are involved in resistance of the hamster to amebic infection of the liver and spread of amebae in that organ.

Immunopathology

Release of soluble immune complexes in the host would suggest a possible immunopathologic mechanism in the pathogenesis of amebiasis (Aust-Kettis et al. 1981). Kagan (1973) briefly mentioned that circulating immune complexes have been found in patients with amebic liver abscess, but Ortiz-Ortiz et al. (1974) were unable to confirm these findings. Antigenemia in amebic liver abscess has been documented (Ruiz Castañeda et al. 1976, Ganguly et al. 1980). Entamoeba histolytica itself can activate complement by the alternative pathway (Ortiz-Ortiz et al. 1978, Huldt et al. 1979), but the crucial experiments to demonstrate C components associated with antigen-antibody complexes in a suspect lesion have not been done.

Autoimmunity induced by the parasite may also be involved in amebiasis. Faubert et al. (1978) discovered autoantibodies against human liver in the sera of ALA patients, who also had antiamebic antibodies detectable by IHA. When rabbits are immunized with whole cell amebic antigen, they not only develop antibodies against E. histolytica but also against rabbit liver cells, particularly intracellular components. Faubert et al. (1978) concluded that an animal model for hepatic amebiasis is needed to understand this phenomenon.

Vaccines

Encouraged by successful vaccines against amebiasis in hamsters and guinea pigs, Sepúlveda (1980b) set the goal on development of a vaccine for use in man. Swartzwelder and Muller (1950) first attempted a vaccine by partially protecting rats against intracecal challenge infection following repeated subcutaneous injections with amebic antigen without adjuvant. The work of Krupp (1966, 1976) further suggested the feasibility of a vaccine when she showed that various strains of E. histolytica share common antigens, which stimulate a humoral response in human populations from widely separate geographic areas. After injections of high molecular weight proteins from axenic amebae in complete Freund's adjuvant (CFA), guinea pigs are protected against intracecal challenge infection with pathogenic E. histolytica (Krupp 1974b, Vinayak et al. 1980). Unfortunately, lack of an adjuvant control group made the specific contribution of amebic antigen impossible to assess.

Other workers have sought a vaccine to protect against amebic liver abscess. In better-controlled studies, hamsters were protected against intrahepatic inoculation with axenic amebae following immunization by various means: live monoxenic or axenic amebae of a homologous strain given

i.d., subcutaneously (s.c.), or intramuscularly (i.m.) (Sepúlveda et al. 1973b, Ghadirian and Meerovitch 1978b); axenic antigen plus CFA given s.c. or antigen alone given i.p. (Sepúlveda et al. 1971b); high molecular weight fraction of axenic antigen with CFA given i.m. (Ghadirian et al. 1980). Sepúlveda et al. (1978) developed improved vaccines using ribosomal or lysosomal fractions of axenic E. histolytica (Arroyo Begovich 1978) that confer complete protection to newborn hamsters against intrahepatic challenge infection (Ortíz-Ortíz 1978). Experiments with the lysosomal antigen have been extended to monkeys (Sepúlveda 1980b).

Successful vaccination against intracecal and intrahepatic infection with E. histolytica has been achieved in animals. Not all the results of immunization experiments are positive, however. Immunization of hamsters with axenic HK-9 antigen does not protect them against intrahepatic challenge infection with monoxenic HM2:IMSS amebae whether or not adjuvant is used (Tanimoto-Weki et al. 1973b). Therefore, axenic amebic antigen does not protect hamsters against hepatic amebiasis caused by highly pathogenic amebae of a heterologous strain grown monoxenically.

Standard methods for manufacturing amebic vaccines and a standard animal model for evaluating them are sorely lacking. Even if a vaccine suitable for use in man can be developed, the need remains to understand the immunologic determinants of disease in amebiasis.

Animal Models for Amebiasis

Prior to a review of animal models for amebiasis, it is apropos to discuss animal models in general. Animal models have certain advantages over clinical studies in man (Smith 1973). It is often impractical or unethical to apply selectively some interventions in clinical studies. Animal models provide an opportunity for uniformity and control over experimental variables such as age and sex of animal, pathogenicity and dose of organism, or time and route of infection. Models make possible a large number of observations and sampling strategies to study pathogenesis over time. Mitruka et al. (1976) emphasized the need for more than one model for a given disease so that comparative studies among various hosts might explain the disease process at different levels. Of course, the risk in using any model is that it may not accurately reflect the disease process as it occurs in man (Smith 1973).

Animal models should meet certain criteria to be useful. If the disease being studied is infectious, the animal must

be susceptible to the organism but free from infection with interfering organisms (Singer 1965, Frenkel 1972). The disease process in a model must be analogous to that in man (Frenkel 1972) and ideally should reflect the human spectrum of disease. The advantages are obvious in choosing a model that has been thoroughly studied with regard to its anatomy, physiology, biochemistry, immunology, genetics, and naturally occurring diseases (Mitruka et al. 1976). Gill (1980) discussed the selection of animals with different types of genetic background for experimental purposes. Inbred strains are the most suitable animal models for studying pathologic processes and immune mechanisms.

Practical considerations are just as important as biological ones. The animal must be available in sufficient quantity of the right age, size, and sex. Purchase costs and variable costs related to animal husbandry may be limiting factors. The animal should be easy to feed on a readily available, low cost diet. Investigators are wise to choose an animal that is easy to handle and requires little cage space. Overall, a model should be the most suitable animal for the experiment (Mitruka et al. 1976).

The uses of animal models are varied. Most animal models have been selected to reflect pathophysiologic changes in man (Mitruka et al. 1976). Disease processes in

useful models should resemble the disease in man, but identical pathological changes are unnecessary for validity. For example, it is sometimes beneficial to focus on an exaggerated immune response or anergy in the experimental animal. Animals are used to study pathogenesis of disease and pathogenicity of disease-causing organisms; they are employed to screen putative toxic, mutagenic, teratogenic, or carcinogenic substances. Preventive and therapeutic measures have been evaluated or invented in animals. Naturally occurring models have been found for a number of biochemical defects and immunodeficiencies in man (Leader and Padgett 1980), but inherited disorders can be more easily studied with inbred animals. Basic biologic mechanisms, nutritional diseases, and epidemic models are appropriate subjects for animal experimentation. Likewise, most of our knowledge about host-parasite relationships came from animal studies. Homburger (1972) mentioned most of these types of research in discussing Syrian hamsters as models. This incomplete list of experimental types suitable for animal models should also include testing hypotheses that originate from prior work done either in vivo or in vitro.

Primates

Miller (1952) summarized host-parasite relationships between primates and E. histolytica in the following manner: (1) New World monkeys rarely harbor E. histolytica in nature but are susceptible to infection, and tissue invasion usually leads to amebic dysentery; (2) Macaca representing Old World monkeys often harbors the parasite in nature and can be readily infected experimentally; but tissue invasion is infrequent and dysentery, rare; (3) man can have a high infection rate but infrequently experiences dysentery. In their host-parasite relationship with E. histolytica the Old World monkeys most closely resemble man.

Monkeys have been used experimentally in some landmark papers on amebiasis. In 1931 Dobell proved that Entamoeba histolytica from monkeys is identical to that from man. A paper by Knowles and Das Gupta (1934) has often been cited as confirmation of Dobell's findings. They used feces from a rhesus macaque containing cysts of E. histolytica for experimental infection of a human volunteer. Apparently, the authors were dealing with Entamoeba hartmanni instead of E. histolytica because they stated, "The strain of Entamoeba histolytica is a small one, with cysts down to 6 to 7 microns in diameter." Miller (1952) experimentally infected Macaca mulatta with human strains of E.

histolytica, but none of the macaques exhibited clinical or pathological evidence of tissue invasion by amebae. Similar results were obtained by Powell and Elsdon-Dew (1961) in baboons (Papio sp.) and by Elsdon-Dew and Maddison (1965) in vervet monkeys (Cercopithecus aethiops pygerythrus). The experiment with baboons was an attempt to show that bacteria are responsible for E. histolytica becoming pathogenic as Westphal (1937) had done in man.

Much has been learned about the pathology of amebiasis in subhuman primates by studying outbreaks of the disease in colonies. Eichhorn and Gallagher (1916) reported such an outbreak among 15 spider monkeys (Ateles). Autopsies revealed extensive amebic ulceration of the intestinal mucosa in colon and rectum and typical ALA in two of the monkeys. More than 60 years later Amyx et al. (1978) remarked about the particular susceptibility of spider monkeys in developing clinical amebiasis while other species of primates in the same colony were also at risk of developing the disease but did not. Miller and Bray (1966) felt that gross and microscopic descriptions of typical lesions in amebic dysentery and ALA are the same in man and the chimpanzee (Chimpansee troglodytes). They believed the chimpanzee model most closely approximates the disease as it

occurs in man. Anderson et al. (1953) considered the pathology of naturally occurring amebiasis in monkeys quite similar to the pathology of human amebiasis, both intestinal and extra-intestinal.

In preparation for testing the efficacy and safety of a vaccine for amebiasis, Mexican workers investigated monkey models for ALA (Sepúlveda 1980b). Intrahepatic inoculation of axenic HM-1 trophozoites was successful in Ateles paniscus (spider monkey) and Cercopithecus sabeus (green monkey) but not in Saimiri sciureus (squirrel monkey) or Erythrocebus patas (patas monkey). One of the green monkeys developed multiple abscesses in the liver under immunosuppression with prednisone and azathiopine (Martínez-Reyes et al. 1980).

Subhuman primates, particularly spider monkeys and chimpanzees, provide excellent models for amebiasis. It would be difficult to rationalize their use, however, because small animals such as the guinea pig for intestinal amebiasis and the hamster for amebic liver abscess are also good models for most purposes. Final testing of an ameba vaccine before a clinical trial in man might be a possible exception.

Rabbit, Oryctolagus

The rabbit has been largely ignored as a model for amebiasis although early studies on experimental intestinal amebiasis were very encouraging. Tobie (1949) pioneered the use of rabbits in experimental amebiasis by infecting them with cysts per os or by inoculating amebae into the cecum. Gross and microscopic pathology of amebic lesions in the rabbit intestine is quite similar to human amebiasis (Tobie 1949, Hunninen and Boone 1957). Lushbaugh et al. (1979) demonstrated an enterotoxin from Entamoeba histolytica, which induces fluid secretion in ligated rabbit ileal loops.

Candreviotis (1966) produced necrotic ulcers in the liver of one rabbit by intraperitoneally injecting axenic 200:NIH amebae provided by Diamond (Diamond et al. 1973). The orbital venous plexus is an unsuccessful route of infection for monoxenic amebae (Westphal and Michel 1970). Susceptibility of rabbits to hepatic amebiasis has hardly been explored.

The rabbit probably merits further study as a model for invasive intestinal amebiasis. Experiments on pathogenesis may be fruitful because of the close similarity between amebic intestinal lesions in the rabbit and in man. Large

size of the rabbit is an advantage for any required surgical procedures, but higher costs for purchase and husbandry are disadvantages.

Jird, Meriones unguiculatus

Diamond et al. (1974b) used the jird as a model for hepatic amebiasis. Meriones unguiculatus is commonly called the gerbil. When jirds and hamsters were inoculated intrahepatically with axenic HM-1 trophozoites, they proved equally susceptible to infection. Severity of lesions was greater in hamsters because half of them died; jirds survived. No sex difference in severity of lesions was seen in either model. The two models differ somewhat in their histopathologic responses to the parasite. Lesions in jirds have a firmer consistency and mostly discrete, small, multiple abscesses whereas hamsters have confluent lesions. Although amebae are more numerous in jird lesions, fibrosis about the lesions is more marked in the jird.

Direct comparison of jirds with hamsters is beneficial because the hamster is widely regarded as the best model for hepatic amebiasis (World Health Organization 1969). Jirds have also been used to assess the pathogenicity of axenic amebae in the same fashion as hamsters (Diamond et al. 1973). The potentiated fibrotic response of the jird makes

it a valuable model because jirds seem to contain hepatic amebiasis more easily than man.

Norway Rat, Rattus norvegicus

The rat has been used extensively to test the efficacy of drugs against E. histolytica (Neal 1951). Jones (1946) first developed a reproducible method for infecting rats and scoring the severity of intestinal lesions. The course of experimental intestinal amebiasis in the rat is fulminant, but self curing (Jones 1948); and clinical signs of infection are usually absent even in cases of massive ulceration (Jones 1946). Entamoeba histolytica occurs naturally as a commensal in the rat but sometimes invades the cecal wall and produces superficial ulcers without clinical signs (Hoare 1959). Care must be taken to differentiate E. muris from E. histolytica in experimental rats (Neal 1949). Weanling rats are susceptible to infection by amebic cysts per os, and diet plays a role in susceptibility of rats to amebic infection (Thompson 1959).

Stewart and Jones (1948) and Dutta and Srivastava (1974) described the pathology of cecal amebiasis in rats. The pathological changes are not unlike those observed in man although acute inflammation seems heightened around areas of tissue invasion.

Neal (1956) used the rat to study pathogenicity of E. histolytica and showed that invasiveness is a property of the ameba apart from its bacterial associates. Furthermore, amebae cultured from asymptomatic cyst passers are not invasive in the rat cecum although amebae from patients with symptomatic intestinal amebiasis produce ulcers in the rat (Neal and Vincent 1955). Jones' (1946) method for infecting rats and Neal's (1951) system for scoring severity of cecal lesions have been adopted by others who used the rat model to study pathogenicity of various ameba strains (Healy and Gleason 1966, Soh et al. 1969, Wang and Cross 1974). Some investigators preferred different methods but still chose the rat model (Schensnovich and Soloviev 1963, Rao and Padma 1971, Singh et al. 1971).

There are two published papers on attempts to establish intestinal infections with E. histolytica in inbred rats. Neal and Harris (1977) found that PVG/C and A/GUS inbred strains of rat are less susceptible to intracecal inoculation with pathogenic Biswas strain amebae than Wistar/WRL rats, the model long used for drug screening (Neal 1951). The same strain of ameba is not infective when inoculated intracecally into inbred WAG rats (Bray and Harris 1977a). Germfree Wistar rats are also refractory to intracecal infection with axenic HK-9 amebae (Phillips et al. 1972).

Axenic strain HK-9 is relatively nonpathogenic for hamster liver (Diamond et al. 1974a).

Despite its success in chemotherapeutic trials of luminal amebicides, the rat is a poor model for hepatic amebiasis. Williams (1959) abandoned the rat for screening systemic amebicides because very few develop liver lesions containing amebae. Westphal and Michel (1970) were unable to produce amebic infection by inoculating monoxenic amebae into the liver parenchyma of four rats.

Several authors (Stewart and Jones 1948, Kasprzak 1968, Neal and Harris 1977) failed to find amebic liver lesions in rats with invasive intestinal amebiasis, but Ishaq et al. (1980) reported ALA in half of the inbred rats (unspecified strain) that developed cecal infections with strain E.h. 81 amebae.

Weanling rats are quite useful as a model for intestinal amebiasis but not ALA. In many respects, experimental cecal amebiasis in rats is similar to intestinal amebiasis in man (Neal 1951). Successful combinations of axenic amebae and inbred strains of rat should be sought for future efforts.

House Mouse, Mus musculus

So far, the mouse has seen limited usefulness as a model for amebiasis. If inbred murine models for intestinal

and hepatic amebiasis were available, the door would be open to definitive studies on the immunology of the disease.

Unfortunately, most attempts to infect the mouse with E. histolytica have had marginal success. Working in China before a reliable means of culturing E. histolytica existed, Kessel (1923) fed human feces containing amebic cysts to mice and observed that intestinal amebiasis in the mouse is less severe than in kittens. Intracecal inoculation of white mice with pathogenic amebae and associated bacteria produces few intestinal infections with E. histolytica, usually of mild severity (Lancastre et al. 1968, Westphal 1970, Ray and Chatterjee 1981). Results of cecal infection are highly dependent upon the strain of ameba (Westphal 1970). Two methods for manipulating the mouse prior to infection have been used to induce more severe intestinal amebiasis in more animals. Lancastre et al. (1968) used immunological tolerance to injected egg albumen, and Ray and Chatterjee (1981) gave the animals a purgative while starving them. Knight and Warren (1973) discovered a synergistic relationship between bisexual infections with ovipositing Schistosoma mansoni and amebic ulceration of the cecum in the mouse. The presence of Trichuris muris also exacerbates E. histolytica infection of the cecum (Knight and Chew 1974). The significance of these findings is left

to conjecture because the corollary in man has not been documented.

Neal and Harris (1977) investigated four inbred strains of mouse as models for intestinal amebiasis, but the infection rate and severity of lesions (when present) were disappointing in general. Using the Biswas strain of E. histolytica in C3H/mg mice, Wijesundera (1980) was unable to increase the infection rate or severity of cecal lesions by immunosuppressing mice with either cyclophosphamide or antilymphocyte serum. The degree of immunosuppression was not documented by proper controls in this experiment, however. The search should continue for an inbred murine model for intestinal amebiasis.

The mouse is a poor model for intestinal amebiasis because it is difficult to infect without manipulation. There is insufficient information to know whether the disease process in mice is similar to that in man. Westphal (1970) reported necrotic amebic ulcers in the ceca of mice but acknowledged that bacteria could have played a role in the pathologic changes. Axenic amebae have not been used for cecal infection of mice, presumably because they do not survive there. Entamoeba muris can be another confounding organism in experimental intestinal amebiasis (Neal 1949)

because it is a common commensal in the gut of rodents (Neal 1950). Most investigators have carefully distinguished between E. histolytica and E. muris in mice, but Ray and Chatterjee (1981) failed to mention E. muris in their work.

The mouse has also been used for extra-intestinal amebiasis with mixed results. Inoculation of amebacrithidia cultures of pathogenic E. histolytica either intradermally or into the orbital venous plexus was unsuccessful (Westphal and Michel 1970). The same strain of ameba did produce mesenteric abscesses when given i.p. (Westphal 1970, Westphal and Michel 1970). Previous i.p. injection of egg albumen (but not toxic Proteus cultures) exacerbated abdominal amebic abscesses in mice (Westphal and Michel 1970). Although gross lesions were not evident in the brain, E. histolytica could be cultured from the brains of some mice several days following intracerebral injection (Westphal and Michel 1970). Mattern and Keister (1977a) found newborn NIH Swiss mice (specific pathogen free) highly susceptible to intracerebral inoculation with axenic HM-1 amebae. Fewer or no amebae were seen in the brains of mice inoculated with less pathogenic strains.

So far, a murine model for hepatic amebiasis has eluded competent efforts (Westphal and Michel 1970, Gold and Kagan 1978). An observation by Wijesundera (1980) is worth

pursuing, however. When inbred C3H/mg mice were given antilymphocyte serum before intracecal inoculation with pathogenic amebae, all of the mice developed liver lesions by nine days. The histological appearance of these lesions was similar to that in man and experimentally infected hamsters.

The mouse has not been fully exploited as an experimental host for Entamoeba histolytica. The likelihood of developing a murine model for hepatic amebiasis seems greater than for intestinal amebiasis. Nevertheless, one possible approach may be intracecal inoculation of axenic amebae in newborn mice, a successful technique in newborn guinea pigs (Diamond et al. 1978b). The primary emphasis should be a systematic search for an inbred mouse suitable as a model for intestinal or hepatic amebiasis.

Guinea Pig, Cavia porcellus

Carrera and Faust (1949) reviewed the few previous attempts to infect guinea pigs with Entamoeba histolytica and reported on their success with the model. Theirs was a very careful study with regard to experimental conditions, pathology, and natural parasites of the guinea pig. Injection of pathogenic ameba cultures into the terminal ileum produces severe cecal amebiasis in most animals.

Subsequent studies featured direct inoculation of amebae into the cecum (Sadun et al. 1950, Taylor et al. 1950).

Carrera and Faust (1949), Taylor et al. (1950), and Phillips et al. (1955) described the pathology of intestinal amebiasis in the guinea pig. Amebic lesions are generally confined to the cecum and colon. The most striking histopathological changes are necrosis and lysis wherever amebae invade host tissue. In most respects the lesions are similar to intestinal amebic ulcers in man. Finding amebae in lymphoid follicles of the guinea pig submucosa is dissimilar to the human disease (Taylor et al. 1950, Maegraith and Harinasuta 1959). Kaushik et al. (1977) simulated formation of amebic granulomas in the cecal wall of guinea pigs by repeated s.c. injections of amebic antigen prior to intracecal inoculation with amebae.

Except for newborn guinea pigs (Diamond et al. 1978b), axenically cultivated amebae have not been used successfully to establish intestinal infection in this model (Candreviotis 1966, Phillips et al. 1972). Phillips et al. (1972) thought that amebae in their experiment had lost pathogenicity in axenic culture and attributed this to losing the ability to encyst (Phillips 1973). However, at least one of the axenic strains, i.e., HM-1, is pathogenic in hamster liver

(Diamond et al. 1973) and produces cecal lesions in newborn guinea pigs (Diamond et al. 1978b).

Phillips and his associates published a landmark series of papers on the role of bacteria in the etiology and pathogenesis of intestinal amebiasis in the guinea pig. Although large numbers of amebae in the inoculum can induce cecal lesions in germfree guinea pigs (Phillips 1964), bacteria and amebae act synergistically to cause disease (Phillips et al. 1955, Phillips and Wolfe 1959, Phillips and Gorstein 1966). Microorganisms contribute to the pathogenesis and pathology of intestinal amebiasis (Phillips et al. 1955) and determine the severity and prognosis of this disease under experimental conditions (Phillips and Gorstein 1966). Jervis and Takeuchi (1979) reviewed use of the gnotobiotic guinea pig as a model for intestinal amebiasis.

Carrera and Faust (1949) showed that amebae can be cultured from the liver of a guinea pig with invasive intestinal amebiasis when amebae can not be demonstrated histologically in the liver. Rees et al. (1954) confirmed this observation. Therefore, Entamoeba histolytica is able to reach the liver from intestinal foci in the guinea pig although amebae may not be evident by microscopy. Carrera and Sadun (1952) showed that livers of guinea pigs with amebic ulcers of the cecum, but without recognizable liver

lesions, are greatly enlarged. They also observed hepatomegaly in human cases of invasive amebic colitis without evidence of amebic lesions in liver sections.

Although spontaneous amebic liver abscess had not been observed in guinea pigs, the foregoing results encouraged others to try guinea pigs as a model for hepatic amebiasis. Despite numerous attempts using various routes of infection, guinea pigs seem refractory to hepatic amebiasis (Maegraith and Harinasuta 1954a, Williams 1959, Candreviotis 1966, Chaudhuri et al. 1966, Soresco and Panaitesco 1969, Westphal and Michel 1970, Bray and Harris 1977a). Nevertheless, Maegraith and Harinasuta (1954b) successfully produced ALA in guinea pigs by alternately injecting trophozoites intracecally and treating the animals with diiodoquine prior to injecting amebae into the portal vein. Solitary or multiple amebic abscesses form in the liver where amebae persist. These results led Maegraith and Harinasuta (1954b) to postulate that "sensitization" to amebic antigens is required for production of ALA in guinea pigs. Two attempts to substantiate this hypothesis (Maegraith and Harinasuta 1959, Beltran H. et al. 1968) were unconvincing; Bray and Harris (1977a) disputed it. Krupp (1956) was interested in other factors that may predispose to ALA, but she was unable to show that trauma to the liver

of guinea pigs by Toxocara canis larvae results in a greater likelihood of amebae colonizing the liver.

In summary, the guinea pig is a suitable model for intestinal amebiasis despite some minor dissimilarities to man. Newborn guinea pigs are susceptible to intracecal inoculation with axenic amebae, and gnotobiotic guinea pigs have proved their usefulness in a number of studies. The guinea pig is not a good model for hepatic amebiasis.

Domestic Dog, Canis familiaris

Since the dog was the first experimental host for Entamoeba histolytica (Lösch 1875), it has been used to study pathology and host-parasite relationships in intestinal amebiasis. Faust and his associates (1953) reviewed much of the early work with the canine model, and he was the first to make extensive use of the dog. He infected dogs with canine and human strains of E. histolytica and found the dog equally susceptible to both (Faust 1932). Experimentally infected dogs show all the clinical signs of human intestinal amebiasis, and intestinal lesions are histologically similar to those in man. The dog is a useful host when highly pathogenic amebae are inoculated per rectum, but other factors such as diet have a profound effect on the severity of canine amebiasis (Thompson 1959). Swartzwelder (1939) used young dogs to show that internal

autoinfection from cysts of E. histolytica does not occur. Serial passage through dogs enhances the pathogenicity of E. histolytica (Meleney and Frye 1937). Jordan (1967) found that amebiasis is more severe in dogs concomitantly infected with Trichuris vulpis and Ancylostoma caninum. A similar observation was made in mice infected with T. muris prior to amebic infection (Knight and Chew 1974). In a classic paper, Swartzwelder and Avant (1952) described acquired immunity to amebiasis in the dog.

Amebic liver abscess is rare in experimentally (Thompson 1959) or naturally infected dogs (Hoare 1959). However, Harris (1901) reported the first ALA produced in an experimental animal. He injected fresh feces from patients with amebic dysentery into the large intestine of puppies. At autopsy ALA complicated amebic dysentery in two of the animals. Kondo (1939) failed to produce ALA in dogs by circumventing the intestinal infection.

With the availability of smaller animal models for intestinal amebiasis, the dog has become less important although the canine model exhibits many similarities with the disease in man. The dog was not a good model for amebic liver abscess in early studies although the question can be reexamined with axenic amebae.

Domestic Cat, *Felis catus*

Kittens were the primary model for amebic dysentery in early studies, because they fail to harbor a naturally occurring species of *Entamoeba* (Rees 1930). Older cats are less susceptible (Dale and Dobell 1917). Kartulis (1887) and Hlava (1887, cited by Dobell 1938)[1] first demonstrated that cats can be infected by rectum with human feces containing *E. histolytica*. Later, Kruse and Pasquale (1894) accomplished experimental infection of a cat and kitten with material from a human amebic liver abscess. In their classic paper, Quincke and Roos (1893) used pathogenicity in kittens to help differentiate between nonpathogenic *Entamoeba coli* and pathogenic *E. histolytica*. Meleney and Frye (1937) and Meleney et al. (1939) used the kitten to ascertain differences in pathogenicity among strains of *E. histolytica*. Dale and Dobell (1917) published a lengthy rep · on experimental infection of kittens with *E. histolytica* for evaluating putative and known antiamebic drugs. However, amebiasis in the kitten could not be cured with any of the drugs tested. They also compared in kittens the pathogenicity of *E. histolytica* from patients with asymptomatic intestinal amebiasis and amebic dysentery.

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[1] A German abstract of Hlava's Bohemian paper on dysentery was published by Kartulis (Centralblatt für Bakteriologie und Parasitenkunde 1:537-539; 1887).

The clinical course of intestinal amebiasis in kittens is usually fulminant (Dale and Dobell 1917). Kittens do not pass amebic cysts in their feces. Extensive mucosal ulceration occurs in the large intestine where lesions extend into the submucosa and sometimes deep into the muscularis. Cats have the tendency to develop amebic invasion of mesenteric lymph nodes (Wenyon 1912), unlike man (Councilman and Lafleur 1891). Although Dale and Dobell (1917) implicated septic complications due to bacteria in the inoculum or in the gut of kittens as the cause of death, they did not do blood cultures. Sanders (1928) bled kittens from the heart just before death and demonstrated bacteremia in kittens with amebiasis.

Marchoux (1899) first recognized ALA in a cat with intestinal amebiasis. Wenyon (1912), Dale and Dobell (1917), and Sanders (1928) made similar observations. Kondo (1939) had moderate success producing ALA in kittens by injecting millions of amebae and bacteria into the mesenteric veins or liver parenchyma. Carrera (1950) studied the pathology of early liver lesions in two of 183 kittens with experimental amebiasis. Amebic hepatitis was an unfortunate choice of terminology for the lesions because the pathological description was almost identical to that of small, solid amebic liver lesions in man (Councilman and Lafleur

1891, Rogers 1922, Palmer 1938). Motile trophozoites of E. histolytica were demonstrated repeatedly in the liver lesions (Carrera 1950).

As a model for amebiasis, the cat holds more historical interest than anything else. Some marked dissimilarities are obvious when comparing the intestinal disease in kittens with that in man. Early liver lesions are quite similar to those in man, but infected kittens do not live long enough for the lesions to progress beyond small solid ones (Carrera 1950).

APPENDIX 2

NECROPSY FORM

Date _____ Time _____ Date of infection _____ Ear tag _____

I. Gross pathology

A. Liver

1. Wet weight _____ g 2. Size ____ x _____ x _____ mm

3. Lesions

a. Total no. _____ Single _____ Multiple _____ Confluent ____

b. Affected lobes
Rt dorsocaudal _____ Lt dorsocaudal _____
Ventral median _____ Dorsal median _____

c. Size _____ x _____ x _____ mm

d. Distribution
Subcapsular no.___ Parenchymal no.___ Uniform ___ Localized__

e. Shape of lesions _____ f. Color _____

g. Consistency _____

4. Architecture Necrosis _____ Abscess _____ Scarring _____

5. Color of surface _____ Cut surface _____

6. Consistency of surface _____ Cut surface _____

7. Additional gross description:

B. Spleen

1. Wet weight _____ mg 2. Size ____ x ____ x _____mm

3. Lesions _____ 4. Architecture _____

5. Color _____ 6. Consistency _____

7. Additional gross description

C. Adhesions Yes ___ No ___ Location _____ Extent _____

D. Other organs fixed for microscopic examination:
Brain _____ Kidney _____ Lung _____ Caecum _____
Thymus _____ Lymph nodes (specify) _____
Other _____
Gross description:

II. Microscopic description:

APPENDIX 3

EXPERIMENTAL RESULTS FORM

I. Host

Time of infection _____ Date of infection _____

Time of necropsy _____ Date of necropsy _____ Ear tag _____

II. Experimental design _____ Cell number in layout _____

Strain _____ Time _____ Dose _____

III. Body weight: When infected _____ g Necropsy _____ g

IV. Infection

A. Anesthesia:

Sodium pentobarbital ___ ml = ___ mg/100 g i.p. Metofane _____

B. Inoculum via portal vein

1. Control TP-S10-1 _____ ml

2. Axenic culture of Entamoeba histolytica

Strain _____ Age of culture _____ h

3. Viability in trypan blue _____ %

4. Dosage

_____ viable amebae in 0.50 ml inoculum

C. Surgical notes _____

Specimen no. _____

V. Hematology

Erythrocytes _____ x $10^6/mm^3$

Leukocytes _____ $/mm^3$

Microhematocrit _____ %

Neutrophils _____ %
Lymphocytes _____ %
Monocytes _____ %
Eosinophils _____ %
Basophils _____ %
Nucleated RBC _____ %

VI. Intestinal parasites

E. muris _____ S. obvelata ___ G. muris ____ T. muris _____

VII. Clinical chemistry

A. Serum electrophoresis

Total protein _____ g/100 ml A:G _____

Percentage of total protein

Albumin _____ Beta globulin _____

Alpha-1 globulin _____ Gamma globulin _____

Alpha-2 globulin _____

B. Liver function tests

Total bilirubin _____ mg/100 ml

AST _____ mU/ml CPK _____ mU/ml ALD _____ mU/ml

ALT _____ mU/ml LDH _____ mU/ml ALP _____ mU/ml

VIII. Serology

	Antigen	Reciprocal of titer
IHA	_____	_____
CF	_____	_____

Total amount of serum _____ ml

APPENDIX 4

TABLES OF EXPERIMENTAL DATA

LIST OF TABLES

TABLE 55

NUMBER AND SIZE OF LIVER LESIONS IN HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF *Entamoeba histolytica* INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands	Animal No.	No. of Lesions	Size of Largest Lesion		
				Length (mm)	Width (mm)	Depth (mm)
HM-1	50	13	30	7.0	6.0	6.0
		14	26	19.0	13.0	7.0
		15	4	24.0	19.0	11.0
		16	0	. . .	. . .	. . .
	Mean		15.0	16.67	12.67	8.00
	SD		15.19	8.737	6.506	2.646
	125	17	18	4.0	3.0	3.0
		18	8	21.0	19.0	10.0
		19	8	11.0	11.0	7.0
		20	6	37.0	20.0	11.0
	Mean		10.0	18.25	13.25	7.75
	SD		5.42	14.315	7.932	3.594
	315	21	15	15.0	8.0	7.5
		22	5	22.0	18.0	11.0
		23	12	27.0	24.0	8.0
		24	8	30.0	24.0	11.0
	Mean		10.0	23.50	18.50	9.38
	SD		4.40	6.557	7.550	1.887

TABLE 55—Continued

Ameba Strain	Dose* in Thousands	Animal No.	No. of Lesions	Size of Largest Lesion: Length (mm)	Width (mm)	Depth (mm)
HU-21	50	37	2	.7	.3	.3
		38	0	. . .	. . .	. . .
		39	1	1.0	1.0	1.0
		40	1	1.0	.2	.2
	Mean		1.0	.90	.50	.50
	SD		.82	.173	.436	.436
	125	41	6	1.0	1.0	1.0
		42	16	2.0	1.5	1.5
		43	0	. . .	. . .	. . .
		44	0	. . .	. . .	. . .
	Mean		5.5	1.50	1.25	1.25
	SD		7.55	.707	.354	.354
	315	45	0	. . .	. . .	. . .
		46	24	5.0	4.0	4.0
		47	0	. . .	. . .	. . .
		48	0	. . .	. . .	. . .
	Mean		6.0			
	SD		12.00			
200:NIH	50	1	4	3.0	2.0	2.0
		2	0	. . .	. . .	. . .
		3	48	10.0	8.0	8.0
		4	0	. . .	. . .	. . .
	Mean		13.0	6.50	5.00	5.00
	SD		23.41	4.950	4.243	4.243

TABLE 55—Continued

Ameba Strain	Dose* in Thousands	Animal No.	No. of Lesions	Size of Largest Lesion: Length (mm)	Width (mm)	Depth (mm)
200:NIH	125	5	13	.1	.1	.1
		6	2	11.0	8.0	5.0
		7	4	17.0	9.0	9.0
		8	0	. . .	. . .	. . .
	Mean		4.8	9.37	5.70	4.70
	SD		5.74	8.568	4.875	4.458
	315	9	35	9.0	5.0	5.0
		10	1	18.0	18.0	8.0
		11	13	30.0	22.0	12.0
		12	1	6.0	3.0	3.0
	Mean		12.5	15.75	12.00	7.00
	SD		16.03	10.782	9.416	3.916
HK-9	50	25	1	3.0	1.0	1.0
		26	0	. . .	. . .	. . .
		27	0	. . .	. . .	. . .
		28	0	. . .	. . .	. . .
	Mean		.2			
	SD		.50			
	125	29	142	.1	.1	.1
		30	1	.5	.5	.5
		31	2	.2	.2	.2
		32	0	. . .	. . .	. . .
	Mean		36.2	.27	.27	.27
	SD		70.50	.208	.208	.208

TABLE 55—Continued

Ameba Strain	Dose* in Thousands	Animal No.	No. of Lesions	Size of Largest Lesion: Length (mm)	Width (mm)	Depth (mm)
HK-9	315	33	5	3.0	1.0	1.0
		34	0	. . .	. . .	. . .
		35	12	7.0	4.0	4.0
		36	3	1.0	1.0	1.0
	Mean		5.0	3.67	2.00	2.00
	SD		5.10	3.055	1.732	1.732

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows for each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively. Uninfected control animals were excluded.

TABLE 56

ORGAN WEIGHTS AND CHANGE IN BODY WEIGHT OF UNINFECTED CONTROLS AND HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF *Entamoeba histolytica* INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands	Animal No.	Change in Body weight (g)	Liver Weight (g)	Spleen Weight (mg)
HM-1	Control	49	-5.7	4.69	68.2
		54	- .4	4.22	81.0
		51	-1.4	4.78	69.8
		52	6.1	4.64	65.0
	Mean		- .35	4.582	71.00
	SD		4.876	.2485	6.959
	50	13	-8.3	4.55	181.8
		14	.7	6.65	307.2
		15	-2.7	9.04	361.2
		16	9.9	5.08	82.0
	Mean		- .10	6.330	233.05
	SD		7.630	2.0147	125.649
	125	17	-5.2	2.53	33.8
		18	-1.8	10.38	633.6
		19	2.2	6.83	393.0
		20	1.2	15.68	698.0
	Mean		- .90	8.855	439.60
	SD		3.333	5.5681	300.695

TABLE 56—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Change in Body weight (g)	Liver Weight (g)	Spleen Weight (mg)
HM-1	315	21	-3.1	5.13	179.8
		22	-2.0	9.07	500.6
		23	-9.0	10.02	325.4
		24	.7	12.21	429.8
	Mean		-3.35	9.108	358.90
	SD		4.091	2.9597	139.409
HU-21	Control	53	-7.2	3.25	64.8
		64	.5	4.20	84.8
		55	1.5	4.86	61.2
		60	3.8	4.78	60.8
	Mean		- .35	4.272	67.90
	SD		4.771	.7424	11.409
	50	37	-4.4	3.55	155.6
		38	-1.2	4.33	60.8
		39	-1.0	4.69	79.4
		40	1.9	4.05	74.2
	Mean		-1.18	4.155	92.50
	SD		2.575	.4809	42.790
	125	41	-2.3	2.82	81.0
		42	- .1	4.15	85.0
		43	4.2	4.13	77.2
		44	4.0	4.29	61.6
	Mean		1.45	3.848	76.20
	SD		3.190	.6887	10.241

TABLE 56—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Change in Body weight (g)	Liver Weight (g)	Spleen Weight (mg)
HU-21	315	45	-3.1	4.15	105.4
		46	-17.7	2.53	120.4
		47	6.4	4.34	82.2
		48	4.2	4.32	67.2
	Mean		-2.55	3.835	93.80
	SD		10.886	.8742	23.694
200:NIH	Control	57	-1.8	6.79	189.4
		58	3.3	4.18	59.6
		59	3.0	4.62	71.8
		56	1.1	3.59	50.8
	Mean		1.40	4.795	92.90
	SD		2.345	1.3953	64.907
	50	1	-6.7	4.25	120.8
		2	4.5	3.83	81.6
		3	-3.0	5.87	249.4
		4	1.7	4.48	72.8
	Mean		- .88	4.608	131.15
	SD		4.965	.8836	81.548
	125	5	-5.8	2.96	38.4
		6	-1.8	4.77	162.2
		7	-13.1	8.82	488.6
		8	2.7	4.54	58.2
	Mean		-4.50	5.272	186.85
	SD		6.703	2.4981	208.366

TABLE 56—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Change in Body weight (g)	Liver Weight (g)	Spleen Weight (mg)
200:NIH	315	9	-6.7	3.57	132.8
		10	-17.1	5.83	273.8
		11	-7.0	13.13	330.8
		12	.3	4.16	72.8
	Mean		-7.62	6.672	202.55
	SD		7.161	4.4101	120.034
HK-9	Control	61	-8.3	4.33	169.6
		50	-1.2	4.20	113.8
		62	-3.7	5.57	119.2
		63	-2.6	6.39	100.4
	Mean		-3.95	5.122	125.75
	SD		3.075	1.0466	30.283
	50	25	-1.4	3.57	99.6
		26	-2.9	3.94	108.0
		27	-5.8	3.99	102.8
		28	.6	3.88	57.4
	Mean		-2.38	3.845	91.95
	SD		2.696	.1888	23.292
	125	29	-6.8	3.22	85.8
		30	- .4	4.44	93.2
		31	-9.8	3.88	77.0
		32	9.7	5.10	72.0
	Mean		-1.82	4.160	82.00
	SD		8.626	.8008	9.396

TABLE 56--Continued

Ameba Strain	Dose* in Thousands	Animal No.	Change in Body weight (g)	Liver Weight (g)	Spleen Weight (mg)
HK-9	315	33	-4.0	3.42	54.8
		34	-1.7	4.31	81.4
		35	4.6	5.03	81.0
		36	1.2	4.28	68.0
	Mean		.02	4.260	71.30
	SD		3.719	.6586	12.639

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows for each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively.

TABLE 57

HEMATOLOGY VALUES FOR UNINFECTED CONTROLS AND HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF Entamoeba histolytica INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hematocrit (%)	WBC Count (cells per cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
HM-1	Control	49	7.29	50.0	5614	18	75	3	4	0
		54	6.70	51.0	3449	42	55	3	0	0
		51	6.89	52.5	5807	27	70	3	0	0
		52	7.74	58.0	5251	66	34	0	0	0
	Mean		7.155	52.88	5030.2	38.2	58.5	2.2	1.00	0
	SD		.4610	3.568	1079.07	20.98	18.41	1.50	2.00	0
	50	13	6.43	46.5	8415	67	29	4	0	0
		14	6.72	41.5	8535	39	49	7	5	0
		15	5.86	40.0	4643	32	56	4	7	1
		16	7.04	54.5	5444	26	68	5	1	0
	Mean		6.512	45.62	6759.2	41.0	50.5	5.0	3.2	.2
	SD		.5013	6.537	2008.58	18.13	16.34	1.41	3.30	.50

TABLE 57--Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hematocrit (%)	WBC Count (cells per cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
HM-1	125	17	5.19	34.0	4895	59	30	11	0	0
		18	5.98	39.5	9086	61	33	2	4	0
		19	5.90	41.0	7086	35	56	3	5	1
		20	5.19	37.5	5982	61	38	1	0	0
	Mean		5.565	38.00	6762.2	54.0	39.2	4.2	2.2	.2
	SD		.4342	3.028	1788.86	12.70	11.64	4.57	2.63	.50
	315	21	6.39	48.0	10668	67	28	3	1	1
		22	6.07	38.5	16090	65	25	3	6	1
		23	4.38	28.0	10243	80	16	3	1	0
		24	6.19	42.0	3534	30	60	2	8	0
	Mean		5.758	39.12	10133.8	60.5	32.2	2.8	4.0	.5
	SD		.9278	8.390	5142.34	21.39	19.19	.50	3.56	.58

TABLE 57--Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hematocrit (%)	WBC Count (cells per cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
HU-21	Control	53	6.80	43.0	6080	61	33	6	0	0
		64	6.83	50.5	3715	43	53	3	1	0
		55	6.92	53.0	5106	31	65	3	1	0
		60	7.75	57.0	3217	29	68	1	2	0
	Mean		7.075	50.88	4529.5	41.0	54.8	3.2	1.0	0
	SD		.4529	5.893	1306.71	14.70	15.88	2.06	.82	0
	50	37	6.72	46.5	8921	42	57	1	0	0
		38	7.61	52.5	5319	29	69	2	0	0
		39	7.57	52.0	3970	62	36	1	1	0
		40	7.52	51.5	3013	38	59	2	1	0
	Mean		7.355	50.62	5305.8	42.8	55.2	1.5	.5	0
	SD		.4249	2.780	2589.15	13.94	13.87	.58	.58	0

TABLE 57--Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hemat-ocrit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
HU-21	125	41	7.08	49.5	6469	38	60	1	1	0
		42	7.39	53.0	4655	30	65	2	3	0
		43	7.41	52.0	4847	56	40	3	1	0
		44	7.65	52.5	3875	35	60	2	3	0
	Mean		7.382	51.75	4961.5	39.8	56.2	2.0	2.0	0
	SD		.2337	1.555	1089.36	11.32	11.09	.82	1.15	0
	315	45	7.21	50.5	5360	29	65	5	1	0
		46	6.31	42.0	8122	80	17	1	2	0
		47	6.72	50.5	6259	35	61	1	3	0
		48	7.58	54.0	3869	40	58	0	2	0
	Mean		6.955	49.25	5902.5	46.0	50.2	1.8	2.0	0
	SD		.5558	5.107	1777.89	23.11	22.35	2.22	.82	0

TABLE 57--Continued

Ameba Strain	Dose[a] in Thousands	Animal No.	RBC Count (millions per cu mm)	Hemat-ocrit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
200:NIH	Control	57	7.57	50.5	6743	53	43	3	1	0
		58	6.88	52.5	2952	34	58	3	5	0
		59	7.04	54.0	4925	27	69	4	0	0
		56	6.73	50.0	4870	32	66	2	0	0
	Mean		7.055	51.75	4872.5	36.5	59.0	3.0	1.5	0
	SD		.3659	1.848	1548.10	11.39	11.63	.82	2.38	0
	50	1	6.68	44.0	5510	19	75	6	0	0
		2	6.67	51.0	5794	29	62	5	4	0
		3	6.28	43.5	16114	71	23	2	4	0
		4	7.83	54.0	5137	37	55	4	4	0
	Mean		6.865	48.12	8138.8	39.0	53.8	4.2	3.0	0
	SD		.6698	5.202	5323.64	22.57	22.11	1.71	2.00	0

TABLE 57—Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hematocrit (%)	WBC Count (cells per cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
200:NIH	125	5	5.85	40.5	4056	54	32	14	0	0
		6	7.14	51.0	10950	33	44	3	20	0
		7	7.53	43.0	18134	78	11	6	5	0
		8	7.91	52.5	5476	42	54	0	4	0
	Mean		7.108	46.75	9654.0	51.8	35.2	5.8	7.2	0
	SD		.8953	5.895	6387.05	19.50	18.50	6.02	8.77	0
	315	9	6.52	45.0	9628	74	23	2	1	0
		10	6.04	38.5	23391	74	15	8	3	0
		11	5.62	37.0	6800	33	60	7	0	0
		12	6.79	47.5	4748	39	58	1	2	0
	Mean		6.242	42.00	11141.8	55.0	39.0	4.5	1.5	0
	SD		.5181	5.050	8407.66	22.08	23.34	3.51	1.29	0

TABLE 57--Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hemat-ocrit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
HK-9	Control	61	6.45	50.5	5870	28	68	3	1	0
		50	6.92	47.5	4744	52	45	2	1	0
		62	7.47	52.0	3025	28	68	3	1	0
		63	7.01	50.5	3155	44	52	1	3	0
	Mean		6.962	50.12	4198.5	38.0	58.2	2.2	1.5	0
	SD		.4180	1.887	1361.06	12.00	11.62	.96	1.00	0
	50	25	5.77	40.0	8703	54	43	3	0	0
		26	6.76	49.0	5542	45	54	1	0	0
		27	7.15	52.0	10255	36	57	5	2	0
		28	7.13	52.0	2297	43	57	0	0	0
	Mean		6.702	48.25	6699.2	44.5	52.8	2.2	.5	0
	SD		.6470	5.679	3529.75	7.42	6.65	2.22	1.00	0

TABLE 57—Continued

Ameba Strain	Dose* in Thousands	Animal No.	RBC Count (millions per cu mm)	Hemat-ocrit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
HK-9	125	29	6.14	38.0	5377	35	59	6	0	0
		30	7.77	53.0	6378	26	70	1	3	0
		31	6.56	51.5	7123	36	62	2	0	0
		32	7.43	49.5	4811	24	71	0	5	0
	Mean		6.975	48.00	5922.2	30.2	65.5	2.2	2.0	0
	SD		.7547	6.819	1029.84	6.13	5.92	2.63	2.45	0
	315	33	6.24	41.0	6878	77	19	4	0	0
		34	7.95	53.0	5565	7	89	4	0	0
		35	7.06	53.0	5097	33	62	2	3	0
		36	6.82	51.0	1895	55	42	1	2	0
	Mean		7.018	49.50	4858.8	43.0	53.0	2.8	1.2	0
	SD		.7106	5.745	2114.77	29.98	29.74	1.50	1.50	0

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows at each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively.

TABLE 58

SERUM PROTEIN ELECTROPHORESIS FOR UNINFECTED CONTROLS AND HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF *Entamoeba histolytica* INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
HM-1	Control	49	7.1	1.30	56.5	16.6	13.8	9.9	3.1
		54	6.0	1.65	62.2	10.2	8.4	14.9	4.3
		51	5.7	2.21	68.9	7.8	6.7	13.1	3.6
		52	5.3	1.42	58.6	10.0	8.7	18.8	3.8
	Mean		6.02	1.644	61.55	11.15	9.40	14.18	3.70
	SD		.772	.4026	5.436	3.793	3.063	3.712	.497
	50	13	5.0	1.01	50.3	10.7	11.2	24.9	3.0
		14	5.0	.78	43.9	11.0	12.5	24.9	7.7
		15	6.2	.77	43.6	10.3	8.5	22.1	15.6
		16	6.8	1.35	57.5	15.8	12.0	10.3	4.4
	Mean		5.75	.979	48.82	11.95	11.05	20.55	7.68
	SD		.900	.2723	6.557	2.583	1.782	6.960	5.639

TABLE 58--Continued

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
HM-1	125	17	5.5	1.16	53.8	10.5	14.9	18.4	2.4
		18	5.0	.76	43.2	12.4	11.4	19.7	13.3
		19	6.2	.87	46.5	9.4	8.3	23.4	12.4
		20	7.2	.36	26.5	4.9	4.2	20.1	44.3
	Mean		5.98	.789	42.50	9.30	9.70	20.40	18.10
	SD		.954	.3326	11.550	3.184	4.551	2.128	18.152
	315	21	5.3	.82	45.0	12.0	10.5	26.9	5.6
		22	5.6	.77	43.5	9.4	9.6	25.2	12.3
		23	4.6	.73	42.1	12.0	10.3	21.1	14.5
		24	8.3	.40	28.6	6.6	7.1	19.5	38.3
	Mean		5.95	.679	39.80	10.00	9.38	23.18	17.68
	SD		1.622	.1896	7.560	2.577	1.565	3.454	14.261
HU-21	Control	53	5.6	1.18	54.2	9.5	14.6	17.9	3.9
		64	6.0	1.66	62.4	10.7	7.9	14.6	4.4
		55	5.8	1.52	60.4	9.5	9.1	15.4	5.7
		60	6.5	1.70	62.9	10.4	9.7	12.1	4.9
	Mean		5.98	1.514	59.98	10.02	10.32	15.00	4.72
	SD		.386	.2346	3.999	.618	2.947	2.390	.768

TABLE 58--Continued

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
HU-21	50	37	7.8	1.14	53.3	17.0	12.9	13.3	3.5
		38	5.3	1.17	54.0	14.9	17.5	9.2	4.5
		39	5.6	1.67	62.6	8.5	7.7	17.7	3.5
		40	5.4	1.90	65.5	8.2	7.8	13.2	5.3
	Mean		6.02	1.471	58.85	12.15	11.48	13.35	4.20
	SD		1.190	.3752	6.127	4.473	4.694	3.472	.872
	125	41	5.2	1.90	65.5	8.5	7.1	14.2	4.7
		42	5.4	1.72	63.3	8.8	7.5	14.7	5.7
		43	5.6	1.57	61.1	9.4	9.8	15.3	4.4
		44	5.7	1.62	61.7	10.4	8.5	14.1	5.2
	Mean		5.48	1.702	62.90	9.28	8.22	14.58	5.00
	SD		.222	.1460	1.966	.838	1.204	.550	.572
	315	45	5.8	1.90	65.5	8.0	8.5	14.6	3.4
		46	5.4	1.27	56.0	11.0	7.6	16.5	8.9
		47	5.9	1.38	58.0	12.0	12.4	12.2	5.4
		48	5.6	1.54	60.5	12.7	7.9	15.6	3.2
	Mean		5.68	1.522	60.00	10.92	9.10	14.72	5.22
	SD		.222	.2733	4.103	2.071	2.232	1.854	2.644

TABLE 58--Continued

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
200:NIH	Control	57	6.4	1.82	64.6	9.4	9.2	13.3	3.5
		58	5.1	1.92	65.8	8.4	8.7	13.6	3.5
		59	7.3	1.39	58.2	19.7	9.8	10.1	2.3
		56	5.1	1.97	66.4	7.6	7.6	16.1	2.4
	Mean		5.98	1.777	63.75	11.28	8.82	13.28	2.92
	SD		1.075	.2657	3.775	5.665	.932	2.461	.665
	50	1	5.1	1.38	57.9	11.5	9.8	17.1	3.6
		2	5.6	1.74	63.6	8.4	7.6	1601	4.5
		3	5.8	.78	43.8	11.4	10.3	23.2	11.4
		4	5.1	1.72	63.3	8.9	8.9	13.8	5.1
	Mean		5.40	1.406	57.15	10.05	9.15	17.52	6.15
	SD		.356	.4509	9.277	1.630	1.185	4.024	3.554
	125	5	5.3	1.07	51.6	8.5	14.3	22.5	3.1
		6	8.4	.87	46.6	14.3	10.4	24.5	4.1
		7	5.3	.71	41.5	10.3	11.2	22.9	14.2
		8	5.6	2.05	67.2	9.3	6.7	13.1	3.7
	Mean		6.15	1.174	51.72	10.60	10.65	20.75	6.28
	SD		1.507	.6010	11.110	2.574	3.125	5.173	5.299

TABLE 58--*Continued*

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
200:NIH	315	9	4.7	.98	49.5	12.4	12.6	20.9	4.6
		10	4.9	.73	42.3	14.5	12.9	21.0	9.3
		11	6.2	.65	39.3	9.8	7.3	19.1	24.4
		12	5.6	1.75	63.6	7.5	8.4	14.9	5.5
	Mean		5.35	1.028	48.68	11.05	10.30	18.98	10.95
	SD		.686	.5025	10.832	3.049	2.867	2.854	9.195
HK-9	Control	61	5.4	1.90	65.5	8.2	7.2	15.0	4.0
		50	6.9	1.43	58.9	13.6	6.7	15.3	5.5
		62	6.3	1.57	61.1	8.7	8.1	15.8	6.4
		63	5.3	1.63	62.0	9.3	7.2	15.0	6.5
	Mean		5.98	1.634	61.88	9.95	7.30	15.28	5.60
	SD		.763	.1982	2.745	2.475	.583	.377	1.158
	50	25	5.6	1.31	56.7	9.6	11.2	19.5	2.9
		26	5.1	1.34	57.2	6.3	9.4	22.8	4.3
		27	5.7	1.04	51.1	9.0	10.2	24.7	5.0
		28	5.8	1.99	66.6	8.4	8.0	13.6	3.4
	Mean		5.55	1.422	57.90	8.32	9.70	20.15	3.90
	SD		.311	.4036	6.425	1.436	1.352	4.867	.935

TABLE 58--Continued

Ameba Strain	Dose* in Thousands	Animal No.	Total Protein (g/100 ml)	A:G	Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
HK-9	125	29	5.1	1.24	55.3	13.5	10.6	15.7	4.9
		30	5.5	1.65	62.3	8.8	7.8	14.6	6.5
		31	5.4	1.33	57.1	7.3	8.2	23.5	3.9
		32	6.0	1.85	64.9	9.4	8.3	13.8	3.5
	Mean		5.50	1.519	59.90	9.75	8.72	16.90	4.70
	SD		.374	.2858	4.463	2.651	1.269	4.468	1.337
	315	33	7.5	1.16	53.8	14.2	18.1	11.1	2.8
		34	5.8	1.66	62.5	7.5	8.1	16.7	5.3
		35	6.4	1.49	59.9	10.4	10.9	15.2	3.6
		36	5.6	2.09	67.6	7.9	7.9	13.1	3.4
	Mean		6.32	1.603	60.95	10.00	11.25	14.02	3.78
	SD		.854	.3863	5.740	3.080	4.768	2.446	1.072

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows for each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively.

TABLE 59

SEROLOGY TITERS FOR UNINFECTED CONTROLS AND HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF *Entamoeba histolytica* INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands		Animal No.	IHA**	CF**
HM-1	Control		49	64	1@
			54	64	1
			51	16	8
			52	16	1
		GMT@@		32.0	1.7
	50		13	2	16
			14	64	64
			15	128	256
			16	16	8
		GMT		22.6	38.0
	125		17	8	16
			18	32	256
			19	64	256
			20	32	2048
		GMT		26.9	215.3
	315		21	8	64
			22	32	256
			23	16	64
			24	16	1024
		GMT		16.0	181.0

TABLE 59--Continued

Ameba Strain	Dose* in Thousands	Animal No.	IHA**	CF**
HU-21	Control	53	32	1
		64	32	32
		55	128	32
		60	8	16
	GMT		32.0	11.3
	50	37	32	4
		38	16	16
		39	32	1
		40	16	16
	GMT		22.6	5.7
	125	41	32	4
		42	64	64
		43	32	32
		44	16	1
	GMT		32.0	9.5
	315	45	32	16
		46	32	256
		47	8	1
		48	8	8
	GMT		16.0	13.4
200:NIH	Control	57	32	1
		58	16	16
		59	16	32
		56	16	8
	GMT		19.0	8.0

TABLE 59--Continued

Ameba Strain	Dose* in Thousands		Animal No.	IHA**	CF**
200:NIH	50		1	16	8
			2	64	8
			3	ND	256
			4	16	1
		GMT		25.4	11.3
	125		5	2	8
			6	8	64
			7	128	128
			8	16	1
		GMT		13.4	16.0
	315		9	4	16
			10	16	128
			11	64	1024
			12	16	16
		GMT		16.0	76.1
HK-9	Control		61	32	1
			50	64	64
			62	16	1
			63	32	1
		GMT		32.0	2.8
	50		25	16	4
			26	32	16
			27	8	32
			28	16	32
		GMT		16.0	16.0

TABLE 59--Continued

Ameba Strain	Dose* in Thousands	Animal No.	IHA**	CF**
HK-9	125	29	8	64
		30	32	128
		31	32	64
		32	8	64
	GMT		16.0	76.1
	315	33	4	8
		34	32	4
		35	32	32
		36	16	64
	GMT		16.0	16.0

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows for each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively.

** IHA = indirect hemagglutination; CF = complement fixation.

@ Reciprocal IHA titers <2 and CF titers <4 were coded as 1.

@@ GMT = geometric mean titer.

TABLE 60

SERUM ENZYMES AND TOTAL BILIRUBIN FOR UNINFECTED CONTROLS AND HAMSTERS INFECTED WITH VARIOUS DOSES OF AXENICALLY CULTIVATED STRAINS OF Entamoeba histolytica INOCULATED VIA THE PORTAL VEIN

Ameba Strain	Dose* in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
HM-1	Control	49	58	24	369	28	215	385	.5
		54	64	41	284	29	280	710	.3
		51	37	34	209	25	293	462	.5
		52	41	22	189	18	213	596	.5
	Mean		50.0	30.2	262.8	25.0	250.2	538.2	.45
	SD		13.04	8.88	81.79	4.97	42.20	143.91	.100
	50	13	592	442	2160	161	464	162	.0
		14	306	249	1760	124	5760	749	.1
		15	31	28	229	19	4820	4	.3
		16	40	24	256	25	426	283	.6
	Mean		242.2	185.8	1101.2	82.2	2867.5	299.5	.25
	SD		265.78	200.59	1005.02	71.23	2823.51	320.70	.265

TABLE 60—Continued

Ameba Strain	Dose[a] in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
HM-1	125	17	82	132	467	60	264	2077	.5
		18	283	259	1460	151	5400	11	.5
		19	64	43	520	141	4260	69	.3
		20	113	48	1051	318	6740	224	.4
	Mean		135.5	120.5	874.5	167.5	4166.0	595.2	.42
	SD		100.39	100.96	471.06	108.29	2791.81	991.92	.096
	315	21	649	540	2680	177	664	140	.1
		22	168	91	1020	115	3980	735	.5
		23	158	122	844	341	7462	29	.3
		24	70	26	572	155	8312	258	.4
	Mean		261.2	194.8	1279.0	197.0	5104.5	290.5	.32
	SD		262.22	233.62	952.01	99.37	3503.73	310.74	.171
HU-21	Control	53	87	76	523	53	254	1768	.5
		64	83	42	464	35	317	1018	.3
		55	64	56	260	35	328	532	.6
		60	52	50	343	27	324	1017	.7
	Mean		71.5	56.0	397.5	37.5	305.8	1083.8	.52
	SD		16.42	14.51	118.39	11.00	34.80	510.36	.171

TABLE 60—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
HU-21	50	37	92	38	589	47	270	1305	.2
		38	25	30	139	20	348	81	.4
		39	31	32	130	126	275	249	.5
		40	41	36	207	29	345	281	.4
	Mean		47.2	34.0	266.2	55.5	309.5	479.0	.38
	SD		30.55	3.65	217.90	48.32	42.79	557.61	.126
	125	41	42	16	173	22	205	322	.2
		42	47	29	134	31	310	981	.5
		43	58	36	296	43	323	674	.5
		44	72	58	324	39	337	1514	.5
	Mean		54.8	34.8	231.8	33.8	293.8	872.8	.42
	SD		13.30	17.58	92.46	9.29	60.19	505.22	.150
	315	45	70	37	295	28	226	456	.3
		46	24	23	141	18	308	89	.4
		47	47	38	260	29	424	346	.2
		48	63	21	221	22	280	655	.4
	Mean		51.0	29.8	229.2	24.2	309.5	386.5	.32
	SD		20.41	9.00	66.14	5.19	83.58	235.99	.096

TABLE 60—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
200:NIH	Control	57	70	49	474	44	133	952	.3
		58	131	48	794	49	348	1868	.3
		59	55	33	242	31	357	655	.4
		56	54	36	209	25	280	422	.3
	Mean		77.5	41.5	429.8	37.2	279.5	974.2	.32
	SD		36.41	8.19	269.95	11.15	103.54	634.08	.050
	50	1	85	66	448	55	165	1509	.1
		2	37	31	184	17	311	111	.2
		3	371	255	798	65	1044	498	.3
		4	254	48	1001	77	327	1444	.3
	Mean		186.8	100.0	607.8	53.5	461.8	890.5	.22
	SD		154.11	104.32	363.28	25.94	394.95	695.36	.096
	125	5	77	67	213	37	239	1291	.5
		6	43	61	209	32	386	415	.6
		7	392	303	1166	76	2030	50	.6
		8	113	71	357	43	306	1520	.5
	Mean		156.2	125.5	486.2	47.0	740.2	819.0	.55
	SD		159.74	118.40	458.37	19.85	861.93	699.71	.058

TABLE 60—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
200:NIH	315	9	217	168	911	105	375	724	.1
		10	157	2	775	56	1620	2	.4
		11	33	16	408	120	1200	11	.4
		12	88	50	313	38	329	1576	.6
	Mean		123.8	59.0	601.8	79.8	881.0	578.2	.38
	SD		80.24	75.41	286.69	39.01	634.72	746.23	.206
HK-9	Control	61	35	20	166	25	168	266	.6
		50	53	37	314	28	322	477	.2
		62	260	147	873	65	148	2720	.3
		63	52	39	415	44	146	1141	.5
	Mean		100.0	60.8	442.0	40.5	196.0	1151.0	.40
	SD		106.99	58.13	304.99	18.34	84.59	1110.46	.183
	50	25	106	37	604	60	267	1685	.3
		26	128	38	743	55	331	1796	.3
		27	71	26	301	50	272	832	.5
		28	65	52	302	32	346	839	.4
	Mean		92.5	38.2	487.5	49.2	304.0	1288.0	.38
	SD		29.78	10.66	222.14	12.20	40.36	524.47	.096

TABLE 60—Continued

Ameba Strain	Dose* in Thousands	Animal No.	Aspartate Aminotransferase (mU/ml)	Alanine Aminotransferase (mU/ml)	Lactate Dehydrogenase (mU/ml)	Aldolase (mU/ml)	Alkaline Phosphatase (mU/ml)	Creatine Kinase (mU/ml)	Total Bilirubin (mg/100 ml)
HK-9	125	29	49	82	295	35	231	1456	.4
		30	32	28	132	25	277	241	.5
		31	39	36	275	26	374	573	.4
		32	60	49	290	28	334	730	.6
	Mean		45.0	48.8	248.0	28.5	304.0	750.0	.48
	SD		12.19	23.80	77.80	4.51	62.87	512.92	.096
	315	33	73	96	289	42	292	1640	.7
		34	64	16	387	33	293	880	.4
		35	172	244	829	64	325	937	.6
		36	57	46	210	28	309	456	.5
	Mean		91.5	100.5	428.8	41.8	304.8	978.2	.55
	SD		54.06	101.20	276.48	15.92	15.59	490.58	.129

* Each row within a dose level for an ameba strain represents observations on one hamster. The four rows for each dose level represent animals necropsied at 3, 7, 11, and 15 days after inoculation, respectively.

TABLE 61

NUMBER AND SIZE OF LIVER LESIONS IN IMMUNOSUPPRESSED HAMSTERS SIX DAYS AFTER INFECTION WITH HM-1 TROPHOZOITES*

Treatment Group**	Animal No.	No. of Liver Lesions	Size of Largest Lesion: Length (mm)	Width (mm)	Depth (mm)
HM-1 control	34	59	8.0	6.0	6.0
	75	30	7.0	6.0	6.0
	26	26	19.0	13.0	7.0
Mean		38.3	11.33	8.33	6.33
SD		18.01	6.658	4.041	.577
Cyclophosphamide	7	0	. . .	. . .	. . .
	10	3	2.5	1.0	1.0
	16	0	. . .	. . .	. . .
Mean		1.0			
SD		1.73			
NRS control	35	3	9.0	6.0	6.0
	41	2	16.0	12.0	9.0
	50	2	6.0	5.0	5.0
Mean		2.3	10.33	7.67	6.67
SD		.58	5.132	3.786	2.082
ALS	3	17	3.0	2.0	2.0
	12	2	5.0	2.0	2.0
	18	6	5.0	5.0	5.0
Mean		8.3	4.33	3.00	3.00
SD		7.77	1.155	1.732	1.732

TABLE 61—Continued

Treatment Group**	Animal No.	No. of Liver Lesions	Size of Largest Lesion: Length (mm)	Width (mm)	Depth (mm)
ATS	1	3	12.0	8.0	6.0
	13	33	5.0	5.0	5.0
	23	1	2.5	.3	.3
	24	35	2.0	1.5	1.5
Mean		18.0	5.38	3.70	3.20
SD		18.51	4.608	3.492	2.731
TX + ALS	15	9	.5	.5	.5
	11	12	2.0	2.0	2.0
Mean		10.5	1.25	1.25	1.25
SD		2.12	1.061	1.061	1.061
TX + ATS	14	3	10.0	8.0	7.0
	22	2	2.0	1.0	1.0
	25	65	2.5	1.5	1.5
Mean		23.3	4.83	3.50	3.17
SD		36.09	4.481	3.905	3.329

* All animals received 50 thousand HM-1 trophozoites via the portal vein.

** NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 62

ORGAN WEIGHTS AND CHANGE IN BODY WEIGHT OF IMMUNOSUPPRESSED HAMSTERS SIX DAYS AFTER INFECTION WITH HM-1 TROPHOZOITES*

Treatment Group**	Animal No.	Change in Body Weight (g)	Liver Weight (g)	Spleen Weight (mg)
HM-1 control	34	-12.3	3.63	131.6
	75	-8.3	4.55	181.8
	26	.7	6.65	307.2
Mean		-6.63	4.943	206.87
SD		6.658	1.5479	90.444
NRS control	35	-16.0	4.14	166.8
	41	-8.3	5.04	308.4
	50	-10.0	5.12	298.0
Mean		-11.43	4.767	257.73
SD		4.045	.5442	78.922
Cyclophosphamide	7	-15.7	4.72	114.2
	10	-15.8	4.86	148.6
	16	-17.5	4.74	133.2
Mean		-16.33	4.773	132.00
SD		1.012	.0757	17.231
ALS	3	-10.1	3.99	327.2
	12	-7.8	5.42	334.4
	18	-8.4	4.98	405.2
Mean		-8.77	4.797	355.60
SD		1.193	.7324	43.105

TABLE 62—Continued

Treatment Group**	Animal No.	Change in Body Weight (g)	Liver Weight (g)	Spleen Weight (mg)
ATS	1	-7.0	5.11	437.6
	13	-14.6	4.62	164.6
	23	-4.0	5.14	331.4
	24	-11.4	3.83	344.4
Mean		-9.25	4.675	319.50
SD		4.686	.6117	113.583
TX + ALS	15	-7.9	4.05	189.4
	11	.8	4.07	330.6
Mean		-3.55	4.060	260.00
SD		6.152	.0141	99.843
TX + ATS	14	-6.5	4.78	242.8
	22	-2.5	3.75	179.8
	25	-4.5	3.80	261.0
Mean		-4.50	4.110	227.87
SD		2.000	.5808	42.610

* All animals received 50 thousand HM-1 trophozoites via the portal vein.

** NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 63

HEMATOLOGY VALUES OF IMMUNOSUPPRESSED HAMSTERS SIX DAYS AFTER INFECTION WITH HM-1 TROPHOZOITES[a]

Treatment Group[aa]	Animal No.	RBC Count (millions per cu mm)	Hemat-ocrit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
HM-1 control	34	7.36	50.0	7759	59	41	0	0	0
	75	6.43	46.5	8415	67	29	4	0	0
	26	6.72	41.5	8535	39	49	7	5	0
Mean		6.837	46.00	8236.3	55.0	39.7	3.7	1.7	0
SD		.4759	4.272	417.71	14.42	10.07	3.51	2.89	0
Cyclophosphamide	7	5.46	34.5	12119	64	31	5	0	0
	10	5.43	35.5	12680	68	26	6	0	0
	16	5.92	35.0	11093	59	37	4	0	0
Mean		5.603	35.00	11964.0	63.7	31.3	5.0	0	0
SD		.2747	.500	804.77	4.51	5.51	1.00	0	0
NRS control	35	6.60	44.5	6376	68	15	17	0	0
	41	7.61	49.0	12252	73	23	2	0	2
	50	6.42	43.5	10125	82	15	3	0	0
Mean		6.877	45.67	9584.3	74.3	17.7	7.3	0	.7
SD		.6414	2.930	2975.08	7.09	4.62	8.39	0	1.15
ALS	3	5.06	35.5	9175	81	9	9	0	1
	12	6.42	42.5	9572	77	15	3	4	1
	18	4.84	36.0	11664	82	8	10	0	0
Mean		5.440	38.00	10137.0	80.0	10.7	7.3	1.3	.7
SD		.8558	3.905	1337.24	2.65	3.79	3.79	2.31	.58

TABLE 63—Continued

Treatment Group**	Animal No.	RBC Count (millions per cu mm)	Hematocrit (%)	WBC Count (cells per cu mm)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
ATS	1	6.02	35.0	4621	87	7	6	0	0
	13	6.14	39.5	18076	84	6	10	0	0
	23	5.81	41.0	4592	85	15	0	0	0
	24	5.64	36.5	3521	80	8	10	0	2
Mean		5.902	38.00	7702.5	84.0	9.0	6.5	0	.5
SD		.2219	2.739	6934.58	2.94	4.08	4.73	0	1.00
TX + ALS	15	6.79	42.0	4253	90	10	0	0	0
	11	6.06	40.5	9676	85	13	1	1	0
Mean		6.425	41.25	6964.5	87.5	11.5	.5	.5	0
SD		.5162	1.061	3834.64	3.54	2.12	.71	.71	0
TX + ATS	14	6.50	46.5	5182	97	2	1	0	0
	22	6.37	43.0	7321	89	6	3	1	1
	25	5.04	38.0	4553	87	7	6	0	0
Mean		5.970	42.50	5685.3	91.0	5.0	3.3	.3	.3
SD		.8080	4.272	1451.02	5.29	2.65	2.52	.58	.58

* All animals received 50 thousand HM-1 trophozoites via the portal vein.

** NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 64

SERUM PROTEIN ELECTROPHORESIS FOR IMMUNOSUPPRESSED HAMSTERS SIX DAYS AFTER INFECTION WITH HM-1 TROPHOZOITES*

Treatment Group**	Animal No.	Total Protein (g/100 ml)	A:G	Pre-Albumin (%)	Albumin (%)	Total Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
HM-1 control	34	5.8	1.62	0	61.7	61.7	8.4	8.7	16.8	4.3
	75	5.0	1.01	0	50.3	50.3	10.7	11.2	24.9	3.0
	26	5.0	.78	0	43.9	43.9	11.0	12.5	24.9	7.7
Mean		5.27	1.136	0	51.97	51.97	10.03	10.80	22.20	5.00
SD		.462	.4304	0	9.016	9.016	1.422	1.931	4.677	2.427
Cyclophosphamide	7	5.8	.87	0	46.4	46.4	14.7	9.5	21.9	7.4
	10	5.1	1.11	0	52.6	52.6	14.5	6.9	22.7	3.2
	16	5.1	.97	0	49.2	49.2	13.8	10.3	23.2	3.6
Mean		5.33	.982	0	49.40	49.40	14.33	8.90	22.60	4.73
SD		.404	.1231	0	3.105	3.105	.473	1.778	.656	2.318
NRS control	35	6.8	.75	1.5	41.3	42.8	22.5	12.6	14.3	7.8
	41	5.7	1.06	5.0	46.6	51.6	10.9	9.6	19.4	8.6
	50	6.7	1.00	5.3	44.8	50.1	9.0	9.5	24.7	6.7
Mean		6.40	.939	3.93	44.23	48.17	14.13	10.57	19.47	7.70
SD		.608	.1677	2.113	2.695	4.708	7.308	1.762	5.200	.954

TABLE 64—Continued

Treatment Group##	Animal No.	Total Protein (g/100 ml)	A:G	Pre-Albumin (%)	Albumin (%)	Total Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
ALS	3	5.7	1.38	4.3	53.6	57.9	12.3	9.7	13.4	6.7
	12	6.7	1.22	4.2	50.8	55.0	7.9	8.7	19.5	8.9
	18	6.3	1.06	2.7	48.7	51.4	10.8	8.1	21.1	8.6
Mean		6.23	1.218	3.73	51.03	54.77	10.33	8.83	18.00	8.07
SD		.503	.1589	.896	2.458	3.256	2.237	.808	4.063	1.193
ATS	1	4.9	.99	5.1	44.7	49.8	9.0	7.4	24.8	9.0
	13	5.9	.94	2.6	46.0	48.6	9.9	10.3	24.1	7.2
	23	5.9	1.37	3.9	53.9	57.8	8.6	7.6	15.1	10.9
	24	5.1	1.29	6.1	50.3	56.4	7.8	9.1	14.5	12.2
Mean		5.45	1.150	4.42	48.72	53.15	8.82	8.60	19.62	9.82
SD		.526	.2132	1.513	4.199	4.623	.873	1.364	5.584	2.188
TX + ALS	15	5.7	1.38	4.3	53.6	57.9	12.3	9.7	13.4	6.7
	11	4.9	1.64	1.7	60.4	62.1	7.9	9.8	14.5	5.7
Mean		5.30	1.507	3.00	57.00	60.00	10.10	9.75	13.95	6.20
SD		.566	.1861	1.838	4.808	2.970	3.111	.071	.778	.707

TABLE 64—Continued

Treatment Group**	Animal No.	Total Protein (g/100 ml)	A:G	Pre-Albumin (%)	Albumin (%)	Total Albumin (%)	Alpha-1 Globulin (%)	Alpha-2 Globulin (%)	Beta Globulin (%)	Gamma Globulin (%)
TX + ATS	14	5.9	1.10	7.2	45.2	52.4	9.3	8.5	23.0	6.8
	22	6.3	1.16	3.3	50.3	53.6	20.9	8.8	11.9	4.7
	25	5.5	1.13	5.7	47.2	52.9	12.0	12.2	18.1	4.7
Mean		5.90	1.128	5.40	47.57	52.97	14.07	9.83	17.67	5.40
SD		.400	.0285	1.967	2.570	.603	6.070	2.055	5.563	1.212

* All animals received 50 thousand HM-1 trophozoites via the portal vein.

** NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

TABLE 65

SEROLOGY TITERS FOR IMMUNOSUPPRESSED HAMSTERS SIX DAYS AFTER INFECTION WITH HM-1 TROPHOZOITES*

Treatment Group**	Animal No.	IHA@	CF@
HM-1 control	34	1@@	16
	75	2	16
	26	64	64
GMT#		5.0	25.4
Cyclophosphamide	7	2	1
	10	2	1
	16	8	1
GMT		3.2	1.0
NRS control	35	16	8
	41	32	32
	50	64	32
GMT		32.0	20.2
ALS	3	ND	16
	12	64	16
	18	32	16
GMT		45.2	16.0
ATS	1	32	8
	13	32	16
	23	16	16
	24	16	8
GMT		22.6	11.3

TABLE 65--Continued

Treatment Group**	Animal No.	IHA@	CF@
TX + ALS	15	4	32
	11	8	8
GMT		5.7	16.0
TX + ATS	14	2	8
	22	8	8
	25	8	1
GMT		5.0	4.0

* All animals received 50 thousand HM-1 trophozoites via the portal vein.

** NRS = normal rabbit serum; ALS = antilymphocyte serum; ATS = antithymocyte serum; TX = neonatal thymectomy.

@ IHA = indirect hemagglutination; CF = complement fixation.

@@ Reciprocal IHA titers <2 and CF titers <4 were coded as 1.

GMT = geometric mean titer.

TABLE 66

NUMBER AND SIZE OF LIVER LESIONS IN HETEROZYGOUS AND ATHYMIC NUDE MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

	9 Days					18 Days				
			Size of Largest Lesion					Size of Largest Lesion		
Treatment Group*	Animal No.	No. of Liver Lesions	Length (mm)	Width (mm)	Depth (mm)	Animal No.	No. of Liver Lesions	Length (mm)	Width (mm)	Depth (mm)
nu/nu HM-1	65	5	3.0	1.0	1.0	66	2	5.0	4.0	4.0
	67	4	2.0	2.0	2.0	72	1	1.0	1.0	1.0
	71	5	3.0	2.0	2.0	68	8	5.0	1.0	1.0
Mean		4.7	2.67	1.67	1.67		3.7	3.67	2.00	2.00
SD		.58	.577	.577	.577		3.79	2.309	1.732	1.732
nu/+ HM-1	36	4	3.0	2.0	2.0					
	42	4	4.0	1.0	1.0	83	3	2.0	1.0	1.0
	57	12	1.5	.8	.8	94	0	. . .	. . .	. . .
Mean		6.7	2.83	1.27	1.27		1.5			
SD		4.62	1.258	.643	.643		2.12			
nu/+ CY HM-1	40	8	2.0	2.0	2.0	41	5	3.2	3.0	2.5
	54	17	9.0	5.0	5.0	70	12	2.0	1.0	1.0
	97	2	.8	.8	.8	98	2	5.0	2.0	1.0
Mean		9.0	3.93	2.60	2.60		6.3	3.40	2.00	1.50
SD		7.55	4.429	2.163	2.163		5.13	1.510	1.000	.866

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide. Uninfected control animals were excluded.

TABLE 67

ORGAN WEIGHTS AND CHANGE IN BODY WEIGHT FOR UNINFECTED CONTROLS AND INFECTED HETEROZYGOUS AND ATHYMIC NUDE MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

Treatment Group*	9 Days				18 Days			
	Animal No.	Change in Body Weight (g)	Liver Weight (g)	Spleen Weight (mg)	Animal No.	Change in Body Weight (g)	Liver Weight (g)	Spleen Weight (mg)
nu/nu control	82	-1.3	2.11	100.6	84	-1.4	1.60	150.0
	90	-1.9	1.62	131.4	85	-2.1	1.70	150.0
	95	-1.2	1.56	112.6	92	-1.9	1.70	150.0
Mean		-1.47	1.763	114.87		-1.80	1.667	150.00
SD		.379	.3017	15.525		.361	.0577	0
nu/nu HM-1	65	2.4	1.62	219.6	66	7.2	2.11	170.0
	67	2.7	1.81	215.4	72	1.1	1.75	98.0
	71	- .6	1.90	194.0	68	6.6	1.62	109.4
Mean		1.50	1.777	209.67		4.97	1.827	125.80
SD		1.825	.1429	13.729		3.362	.2538	38.700
nu/+ control	44	- .4	1.84	146.6	43	-4.4	1.62	94.0
	58	2.1	1.77	131.6	56	- .3	2.64	159.8
	63	- .7	1.26	120.0	61	-3.2	1.73	115.2
Mean		.33	1.623	132.73		-2.63	1.997	123.00
SD		1.537	.3166	13.336		2.108	.5599	33.586
nu/+ HM-1	36	.1	1.78	130.0				
	42	- .9	2.32	287.8	83	-5.4	2.10	150.0
	57	.8	1.98	238.0	94	- .9	1.99	100.0
Mean		.00	2.027	218.60		-3.15	2.045	125.00
SD		.854	.2730	80.669		3.182	.0778	35.355
nu/+ CY HM-1	40	-2.3	1.72	247.2	41	-2.5	1.57	128.2
	54	-3.7	2.26	334.0	70	-5.8	1.67	181.6
	97	.3	1.86	212.8	98	-2.5	1.97	250.0
Mean		-1.90	1.947	264.67		-3.60	1.737	186.60
SD		2.030	.2802	62.459		1.905	.2082	61.054

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide.

TABLE 68

HEMATOLOGY VALUES FOR UNINFECTED CONTROLS AND INFECTED HETEROZYGOUS AND ATHYMIC NUDE MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

Treatment Group*	Time Post Infection	Animal No.	RBC Count (millions per cu mm)	Hemato-crit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
nu/nu control	9 days	82	9.32	48	7495	47.0	46.0	5.5	1.5	0
		90	7.77	44	4936	64.0	32.5	3.0	.5	0
		95	9.85	46	8330	29.5	53.0	15.5	2.0	0
		Mean	8.980	46.0	6920.3	46.83	43.83	8.00	1.33	0
		SD	1.0809	2.00	1768.5	17.251	10.420	6.614	.764	0
nu/nu HM-1	9 days	65	7.51	42	6343	53.5	31.0	11.5	4.5	0
		67	8.14	42	7646	31.5	57.5	7.0	3.0	1.0
		71	8.60	45	7790	46.0	42.5	7.5	4.5	0
		Mean	8.083	43.0	7259.7	43.67	43.67	8.67	4.00	.33
		SD	.5472	1.73	797.12	11.184	13.288	2.466	.866	.577
nu/+ control	9 days	44	9.62	47	4088	45.0	48.0	7.0	0.0	0
		58	9.77	46	4765	13.0	78.0	5.0	4.0	0
		63	9.77	49	2365	18.5	70.0	7.0	4.5	.5
		Mean	9.720	47.3	3739.3	25.50	65.33	6.33	2.83	.17
		SD	.0866	1.53	1237.41	17.110	15.535	1.155	2.466	.289

TABLE 68--Continued

Treatment Group[a]	Time Post Infection	Animal No.	RBC Count (millions per cu mm)	Hemato-crit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
nu/+ HM-1	9 days	36	8.37	48	3996	22.5	72.0	3.5	2.5	.5
		42	9.41	44	5187	43.5	42.5	2.5	11.5	0
		57	9.86	45	8686	34.0	49.0	5.0	12.0	0
		Mean	9.213	45.7	5956.3	33.33	54.50	3.67	8.67	.17
		SD	.7642	2.08	2437.81	10.516	15.500	1.258	5.346	.289
nu/+ CY HM-1	9 days	40	7.77	38	22520	42.0	53.5	4.5	0	0
		54	8.75	44	13177	37.0	45.5	13.5	4.0	0
		97	8.88	46	3615	34.5	45.5	15.0	4.5	.5
		Mean	8.467	42.7	13104.0	37.83	48.17	11.00	2.83	.17
		SD	.6068	4.16	9452.71	3.819	4.619	5.679	2.466	.289
nu/nu control	18 days	84	7.01	44	13001	83.5	16.5	1.0	0	0
		85	7.42	43	12700	74.0	24.5	.5	1.0	0
		92	6.93	48	14562	55.0	32.0	10.0	3.0	0
		Mean	7.120	45.0	13421.0	70.83	24.33	3.83	1.33	0
		SD	.2629	2.65	999.53	14.511	7.751	5.346	1.528	0
nu/nu HM-1	18 days	66	10.21	50	3104	70.0	20.5	5.5	4.0	0
		72	10.31	53	4169	46.5	41.5	9.0	3.0	0
		68	9.17	50	3819	47.0	36.5	9.0	7.5	0
		Mean	9.897	51.0	3697.3	54.50	32.83	7.83	4.83	0
		SD	.6313	1.73	542.82	13.426	10.970	2.021	2.363	0

AD-A128 689 AN EXPERIMENTAL ANIMAL MODEL FOR THE STUDY OF IMMUNITY TO ENTAMOEBA HISTOLYTICA(U) JOHNS HOPKINS UNIV BALTIMORE MD R G TAYLOR 15 APR 83 DAMD17-75-C-5001 6/6

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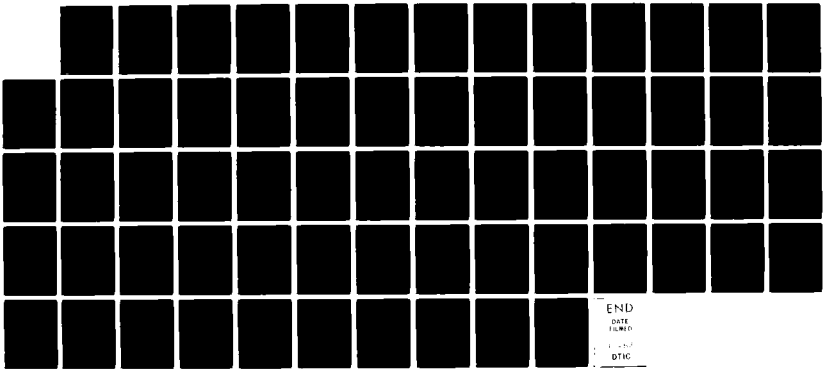

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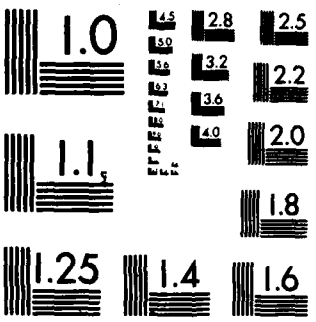

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TABLE 68—Continued

Treatment Group*	Time Post Infection	Animal No.	RBC Count (millions per cu mm)	Hemato-crit (%)	WBC Count (cells per cu mm)	Neutro-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	Eosino-phils (%)	Baso-phils (%)
nu/+ control	18 days	43	9.07	47	4951	28.0	68.0	2.0	2.5	0
		56	7.69	45	8433	20.0	76.0	1.5	2.5	0
		61	8.93	44	3602	9.5	87.0	2.0	2.0	0
		Mean	8.563	45.3	5662.0	19.17	77.00	1.83	2.33	0
		SD	.7596	1.53	2492.75	9.278	9.539	.289	.289	0
nu/+ HM-1	18 days	83	8.04	45	23259	51.0	46.0	1.5	1.5	0
		94	7.77	45	14229	26.0	68.5	1.5	4.0	0
		Mean	7.905	45.0	18744.0	38.50	57.25	1.50	2.75	0
		SD	.1909	0	6385.17	17.678	15.910	0	1.768	0
nu/+ CY HM-1	18 days	41	8.21	44	2470	50.5	39.0	5.5	5.0	0
		70	8.30	44	4654	11.0	78.0	4.0	7.0	0
		98	8.80	46	26810	61.5	34.0	4.0	.5	0
		Mean	8.437	44.7	11311.3	41.00	50.33	4.50	4.17	0
		SD	.3179	1.16	13466.59	26.557	24.090	.866	3.329	0

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide.

TABLE 69

SEROLOGY TITERS FOR UNINFECTED CONTROLS AND INFECTED HETEROZYGOUS AND ATHYMIC NUDE MICE AT NINE AND 18 DAYS AFTER INTRAHEPATIC INOCULATION WITH ONE MILLION HM-1 TROPHOZOITES

Treatment Group*		9 Days			18 Days		
		Animal No.	IHA**	CF**	Animal No.	IHA	CF
nu/nu control		82	1@	1	84	1	1
		90	1	1	85	1	1
		95	1	8	92	1	1
	GMT@@		1.0	2.0		1.0	1.0
nu/nu HM-1		65	1	1	66	1	1
		67	1	1	72	1	4
		71	1	1	68	1	1
	GMT		1.0	1.0		1.0	1.6
nu/+ control		44	1	4	43	1	1
		58	1	4	56	. . . . .	
		63	1	1	61	1	1
	GMT		1.0	2.5		1.0	1.0
nu/+ HM-1		36	4	8			
		42	1	1	83	1	4
		57	1	8	94	1	32
	GMT		1.6	4.0		1.0	11.3
nu/+ CY HM-1		40	1	1	41	1	1
		54	1	32	70	1	16
		97	1	1	98	1	32
	GMT		1.0	3.2		1.0	8.0

* nu/nu = athymic nude mouse; nu/+ = heterozygous mouse; CY = cyclophosphamide.

** IHA = indirect hemagglutination; CF = complement fixation.

@ Reciprocal IHA titers <2 and CF titers <4 were coded as 1.

@@ GMT = geometric mean titer.

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