Experimental Histoplasmosis, I. Methods for Production of Histoplasmosis in Dogs.* (20538)

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The production of histoplasmosis in dogs by respiratory routes of infection has not been reported. De Monbreun(1), who first described histoplasmosis in dogs, reproduced the disease in 3 puppies by the intraperitoneal inoculation of the yeast phase of *Histoplasma capsulatum* in 2 and citrated blood from an infected dog in the third. Feeding of mycelial and yeast phase mixtures produced no marked clinical disease although evidence of infection was observed histologically in 6 dogs. Ruhe and Cazier(2) produced the disease in 4 out of 5 dogs by the severe and unnatural procedure of inoculation of yeast cells directly through the thoracic wall into the lung parenchyma. Cross(3) was unable to produce fatal disease by the intratracheal, oral, intramuscular, or intravenous inoculation of yeast phase *H. capsulatum*. No gross lesions were seen at necropsy of inoculated animals although lesions containing organisms were later demonstrated microscopically.

It is the purpose of this report to describe the comparative effect in dogs of the yeast and mycelial phases of *H. capsulatum* introduced by the respiratory or gastrointestinal routes and the effect of cortisone on infection.

Materials and methods. The 45 dogs used in these experiments were vaccinated against canine distemper. During a pre-inoculation period of at least 2 weeks, all dogs were found to have negative chest x-rays, histoplasmin skin tests and collodion agglutination tests(4). Baseline cultures of blood, urine, feces, gastric washings, and biopsied mesenteric lymph nodes were negative for fungi. Clinical examinations were continued throughout the observation period. Each of the 19 dogs in Exp. I was inoculated (9 gastric and 10 tracheal) with a total of 8 ml of packed mycelial growth.† The first inoculation of one ml was repeated on the 35th day and was followed by 2 ml doses on each of the 75th, 77th, and 79th days. The inocula for 9 dogs (4 gastric and 5 tracheal) and that for 10 dogs (5 gastric and 5 tracheal) were suspended in 5% hog gastric mucin(5) and physiologic saline, respectively. All inocula in these studies were suspended in 3 ml of the respective menstruums.

Each of 16 dogs in Exp. II received 2 ml of packed yeast phase *H. capsulatum*. Eight dogs were inoculated with the organism suspended in saline (4 gastric and 4 tracheal) while the remaining 8 were inoculated with the mucin suspension (3 gastric and 5 tracheal). A third experiment with 18 dogs was designed to study the possible effect of cortisone on infection. Eight animals which had been inoculated with mycelial phase 11 months previously (survivors of Exp. I) were considered to be potentially "immune"; the remaining 10 were healthy dogs not previously inoculated. All 18 dogs received intramuscular injections of 100 mg of cortisone for 16 days. On the 5th day of cortisone administration 16 (8 "immune" and 8 "susceptible") of the 18 dogs were inoculated by the same route and with the same phase of the organism suspended in the same vehicle (saline or 5% mucin) as when they were part of the first experiment. Eight of the 10 "susceptible" dogs were inoculated in the same manner as the 8 "immune" animals. The 2 remaining dogs served as non-infected cortisone treated controls (Table I). Eight of the dogs which survived this latter procedure (6 "immune" and 2 "susceptible") were subsequently chal-

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† Strain G-13, a recent isolate from spontaneous histoplasmosis in a dog, kindly supplied by Charlotte Campbell of the Army Medical Center, Washington, D.C.

TABLE I. Comparative Results of Pathogenicity for Dogs of Mycelial and Yeast Phase *H. capsulatum.*

Route and vehicle	Exp. 1 (mycelial)	Exp. 2 (yeast)	Exp. 3 (mycelial + cortisone)		Exp. 4 (yeast)	
			"Immune"§	"Susceptible"	"Immune"‖	"Susceptible"
Gastric (saline)	0/5*	0/4	0/2	0/2	1/1	
" (mucin)	0/4	0/3	0/2	0/2	2/2	
Tracheal (saline)	0/5	4/4	0/2	1/2†	0/1‡	1/1
" (mucin)	0/5	5/5	0/2	2/2	2/2	1/1
0/2 controls (cortisone)						

* Numerator = No. of dogs with fatal histoplasmosis. Denominator = Total No. inoculated.
† Both dogs acutely ill; spontaneous recovery in one.
‡ Dog acutely ill; became chronic.
§ Previously employed in Exp. 1.
‖ " " " " 1 and 3.

lenged with 2 ml packed yeast cells given intratracheally (Exp. 4).

The dogs inoculated by the gastrointestinal route received the inoculum through a stomach tube. Saline or mucin equal to the volume of inoculum was used to wash adhering material from the sides of the tube into the stomach. The dogs receiving the organisms by the respiratory route were inoculated intratracheally following anesthesia by intravenous injection of sodium thiopental. The dog's head was raised, the mouth opened, and the tongue grasped and pulled forward. A 6-inch, 18 gauge luerlock cannula was inserted through the glottis deep into the trachea. The inoculum was injected into the trachea after attaching a syringe to the cannula. The cannula was then withdrawn and the head was held high for about a minute to allow the material to drain into the bronchi. Cultures of gastric contents, urine, feces, and blood, complete blood counts and collodion agglutination tests were performed weekly for one month after inoculation, biweekly during the second month and monthly during the remainder of the observation period. The detailed hematologic and serologic data will be presented in a separate report. Complete necropsies and postmortem cultures were performed on all dogs which died or were sacrificed.

Results. Exp. 1 (Mycelial phase). None of the 19 dogs inoculated with mycelial phase *H. capsulatum* manifested signs of disease during a 6-month observation period following the primary inoculation regardless of route or menstruum employed (Table I). The 10 dogs inoculated intratracheally developed positive histoplasmin skin tests within one to 3 months. Only one of the 9 dogs inoculated by the gastrointestinal route developed a skin test reaction. Repeated antemortem cultures, as well as postmortem cultures from dogs sacrificed 6 months after the primary inoculation were all negative.

Exp. 2 (Yeast phase). All 9 dogs which received the yeast phase intratracheally developed acute progressive disseminated histoplasmosis in 3 to 5 days and died in from 11 to 42 days following inoculation (Table I). Pyrexia of 1 to 3°F, hyperpnea and partial to complete anorexia were noted 3 to 4 days post-inoculation. Paroxysmal coughing and dyspnea were observed in all animals during the course of the disease. Hyperpnea and dyspnea became more severe as the disease progressed. *H. capsulatum* was isolated from all 9 dogs from both antemortem blood cultures and from the tissues aseptically collected at necropsy. The fungus was isolated frequently from the lung, spleen, and liver, and less often from the mesenteric nodes, bronchial nodes, and heart blood. Extensive pulmonary lesions present in all 9 dogs included discrete cream colored nodules measuring 1 to 3 cm in diameter or complete consolidation of entire lobes, cavitation, liquefaction, emphysema and fibrinous pleuritis (Fig. 1). Granulomatous inflammation of the visceral lymph nodes and liver which were culturally positive for *Histoplasma* presented further evidence of widespread dissemination of the infection. None of the 7 dogs inoculated orally with yeast phase manifested disease during a 6-month post-inoculation period or became sensitive to histoplasmin. Of the 5 dogs sacri-

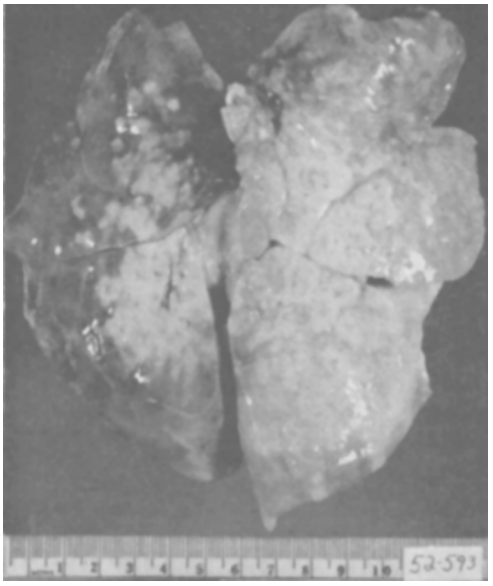

FIG. 1. Lungs (frontal section) from dog which received yeast phase *H. capsulatum* intratracheally. Consolidation of right lung, atelectasis, diffuse and nodular granulomatous pneumonia in left lung.

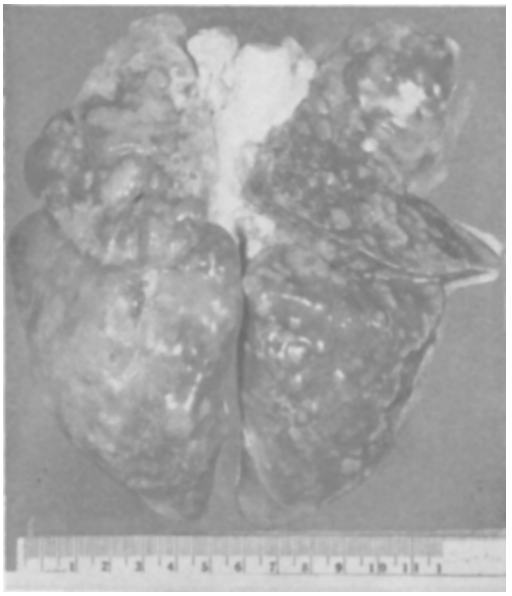

FIG. 2. Lungs from dog which received mycelial phase *H. capsulatum* intratracheally and cortisone. Elevated nodules in all lobes, enlarged bronchial nodes and fibrinous pleuritis.

ficed from this group no evidence of infection was found at necropsy.

Exp. 3 (Mycelial phase plus cortisone). The weight of each dog in this group increased and fluctuated after a week on cortisone treatment due, in part, to changes in the fluid balance. All 4 "susceptible" dogs which were inoculated intratracheally became ill on the 5th, 6th, 12th, and 18th post-inoculation day, respectively. Increased bronchial sounds, dyspnea, and pyrexia were the most consistent signs. Three of the 4 dogs died of acute, progressive, disseminated histoplasmosis between the 18th and 28th day after inoculation with lesions similar to those seen in Exp. 2 (Fig. 2). One developed severe illness, a high histoplasma antibody titer, positive blood cultures, but gradually recovered spontaneously. In contrast, none of the 4 "susceptible" dogs on cortisone which received the mycelial growth by stomach tube showed any signs of histoplasmosis during a post-inoculation period of 2½ months. Similarly negative results were obtained with the 8 "immune" dogs regardless of route of inoculation (Table I). The 2 uninoculated control dogs receiving cortisone only, failed to show signs of infection during this same period.

Exp. 4 (Intratracheal challenge with yeast phase). Eight dogs which had been previously unaffected clinically following inoculation with mycelial phase (Exp. 1) were not affected when challenged with mycelial phase plus cortisone (Exp. 3). To investigate further their relative state of resistance, 6 of these dogs were inoculated with 2 ml of yeast phase organisms, intratracheally. This procedure was followed by severe signs of infection in all 6 with 5 deaths occurring between the 6th to 49th days. Similar fatal infections were obtained with 2 dogs which were unaffected by gastric administration of mycelial phase in Exp. 3 when given yeast phase intratracheally (Table I); both died 10 days after inoculation.

Discussion. H. capsulatum may be grown in either the yeast or mycelial phase. It appears as a yeast in parasitized tissues. The mechanism of natural infection is not yet definitely known. Since severe fatal infections were produced only after intratracheal inoculation, it is possible that the respiratory route is more common, or likely, in spontaneous infections. That the organism was disseminated from the primary pulmonary site of infection, was demonstrated by isola-

tion of the fungus from liver, spleen, mesenteric nodes, heart blood, as well as from the lungs and bronchial nodes. Detailed description of the gross and microscopic pathology, clinical and laboratory data, including serologic results, will be reported in a separate communication.

Little is known about immunity, or reinfection in histoplasmosis. In these studies it has been demonstrated that although intratracheal inoculation with mycelial phase induced no clinical disease a relative immunity to subsequent challenge to mycelial phase. plus cortisone, was observed. However. challenge with yeast phase did produce a severe disease. Since this latter inoculum was large, similar studies with lesser dosages are indicated to clarify the nature of immunity in histoplasmosis.

Summary. Yeast phase *H. capsulatum* induced acute, progressive, fatal histoplasmosis in all of 9 dogs when inoculated intratracheally, but no clinical disease in all of 7 receiving the same inoculum by stomach tube. Mycelial phase produced no clinical evidence of infection in 9 and 10 normal dogs receiving intragastric and intratracheal inoculations, respectively. Cortisone treated dogs, however, were susceptible to intratracheal but not intragastric mycelial phase inoculations. Dogs previously receiving mycelial phase were resistant to reinfection with the same phase even when given cortisone; they succumbed to challenge with intratracheal yeast phase in the absence of cortisone.

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Transfusion of Separated Leukocytes into Irradiated Dogs with Aplastic Marrows. (20539)

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The consistent finding of bone marrow aplasia and agranulocytic ulcerations in dogs dying from lethal doses of x-ray suggested a causal role of neutropenia in death from radiation injury(1,2). To test this thesis, concentrated suspension of homologous white blood cells were transfused into irradiated leukopenic dogs. The method used was not successful in maintaining a normal level of circulating granulocytes. However, significant elevations of the white blood cell counts in dogs with totally aplastic marrows were obtained. This report describes preliminary results on the functional activity and fate of the transfused granulocytes.

Material and methods. Dogs were exposed to whole body irradiation of 600 r (cobalt 60 source, measured in air by a Victoreen rate meter in 4 π geometry), a uniformly lethal dose which resulted in complete bone marrow aplasia. Donor dogs were given an i.m. or s.c. injection of 0.2 to 0.5 ml of a 1:10 solution of turpentine in alcohol which generally elevated their circulating WBC level. All donor dogs were negative for the canine A hemagglutinogen(3). Donor dogs were bled from the femoral artery into siliconed 230 ml bottles, each containing 40 ml of 5% dextran and 0.5% di-sodium ethylenediamine tetraacetate (EDTA) in Hanks' solution. The dextrans† used were 2 lots (No. 237 and No. 649) that

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