A SURVEY TO DETERMINE THE OCCURRENCE OF <u>HISTOPLASMA</u>
<u>CAPSULATUM</u> AND <u>CRYPTOCOCCUS</u> <u>NEOFORMANS</u> IN AIR-CONDITIONERS

by

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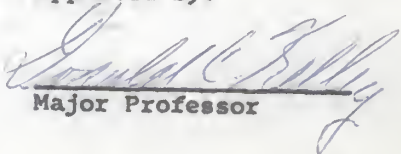
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INTRODUCTION

Cryptococcosis and histoplasmosis are systemic diseases caused by pathogenic fungi. Cryptococcus neoformans, the infectious agent causing cryptococcosis, is a yeast at 25 C and at 37 C. The causative organism of histoplasmosis, Histoplasma capsulatum, is diphasic, having mycelial characteristics at 25 C and yeast characteristics at 37 C.

Since the recovery of H. capsulatum in 1949 and C. neoformans in 1951 as saprophytes from the soil, both organisms have been isolated often. They are recovered with consistency from soil laden with feces of pigeons, starlings, and chickens.

The reservoir of cryptococcosis and histoplasmosis is considered to be the saprophytic occurrence of C. neoformans and H. capsulatum in the external environment. In nature, the mode of transmission is spore-laden dust and the portal of entry is presumably by inhalation.

With the modern trend of air-conditioning and its increasing use in our society, the window air-conditioner could be a reservoir of C. neoformans and H. capsulatum. The window air-conditioner is sufficiently exposed to the atmosphere to make an excellent roost for birds. The fecal matter, deposited by birds while roosting, incorporated with dust may permit the propagation of both C. neoformans and H. capsulatum. If these organisms are present in air-conditioners, they may be a health hazard.

This survey was conducted to determine the presence or absence of the saprophytic occurrence of C. neoformans and H. capsulatum in air-conditioners.

REVIEW OF THE LITERATURE

Sanfelice, in 1894, isolated from peach juice an encapsulated yeast-like fungus which he named Saccharomyces neoformans and with which he produced lesions in experimentally infected animals (Emmons, et al., 1963). Busse and Buschke (1894-1896) independently reported the recovery of a yeast-like organism from a 31 year old woman with a "gumma-like" or "sarcoma-like" lesion of the tibia, who also had lymphadenopathy, and secondary skin lesions. The knee joint subsequently became involved and the patient died with multiple lesions of the lungs, spleen, kidney, bones and skin. Vuillemin, recognizing that the lack of ascospore formation differentiated this organism from the true yeasts, adopted the name Cryptococcus hominis (Littman and Zimmerman, 1956). The naming of the organism was controversial for some time. Authorities have named the organisms, Cryptococcus neoformans, based on usage and priority. As early as 1902, C. neoformans had been recovered from humans, horses, cattle and fruit (Littman and Zimmerman, 1956).

Darling, in 1906, observed a small parasite which he thought to be a protozoan, multiplying in large mononuclear cells of the liver, spleen, lung, lymph nodes and bone marrow. He called the organism Histoplasma capsulatum. Negroni (1965) stated that the second stage in the history of H. capsulatum was accomplished by Da Rocha Lima, revealing the organism to have the characteristics of a budding yeast in lesions in the horse. Using H. capsulatum, De Monbreun (1934) fulfilled Koch's postulates and proved it to be a dimorphic organism. Histoplasmosis, the disease caused by H. capsulatum has occurred in

man (Darling, 1906) and animals (De Monbreun, 1939; Emmons, 1950; Menges and Kintner, 1951).

Cryptococcus neoformans was isolated from milk by Klein (1901) and Carter and Young (1950). However, since its isolation from peach juice, it was not found again as a saprophyte, unrelated to human or animal tissue, until 1951, when it was recovered from the soil (Emmons, 1951).

Emmons (1954) attempted isolation from various specimens of soil and organic debris taken from environments, such as barnyards, granaries, in and around chicken houses and other miscellaneous sources. From the results obtained, Emmons suggested that no significant pattern of distribution was revealed. However, in one collection, 8 of 11 specimens taken from old pigeon nests and droppings yielded C. neoformans. With this information, Emmons suggested that pigeon manure might provide a suitable or preferential medium for the saprophytic growth of C. neoformans. Testing the above hypothesis, Emmons in 1955 reported positive findings of C. neoformans from 63 of 111 specimens of soil collected on 16 farm premises and 3 buildings in the city of Leisburg, Virginia. Only 3 of the 19 premises from which the collections were made failed to yield the organism. Specimens were collected from premises which were selected on the presence of pigeons.

Littman and Borok (1967) stated that C. neoformans had been isolated from beaks and feet but not rectums of 6 of 86 feral pigeons trapped at random in New York. Despite the high isolation of the organism in pigeon excreta and nests, neither the carrier state nor

spontaneous infection has been demonstrated in the pigeon. This is attributed to the high temperature of the bird.

The pigeon is incriminated in the occurrence of histoplasmosis also (Emmons, et al., 1963). Laskowski, et al., (1960) stated that pigeons do not have the infection, but their excreta permitted heavy growth of H. capsulatum in the soil.

Emmons (1949) working with soil samples in and around chicken houses where infected rats had been found isolated H. capsulatum. Zeidburg, et al. (1952) further stated that the organism could best be isolated from soil laden with pigeon and chicken manure. H. capsulatum has also been isolated from soil samples taken from beneath a starling roost (Dodge, et al., 1965).

The organism has been recovered from bats by Shacklette, et al., in 1962. Tesh and Schneidau (1966) inoculated bats intrapertioneally with yeast cells of H. capsulatum. The organism was recovered from the feces of 10 of 12 bats surviving longer than 2 weeks. From this work, Tesh and Schneidau suggested that the bat may serve as a vector for the dissemination of H. capsulatum.

While pigeons, chickens, and starlings have been incriminated in the spread of H. capsulatum, it must be emphasized that they do not become infected (Menges, et al., 1963). It is soil laden with their excreta which harbors the organism.

The mode of transmission of C. neoformans and H. capsulatum is spore-laden dust and the portal of entry is presumably by inhalation of this contaminated dust (Terplan, 1948; Gordon, 1965; Negroni, 1965). Ibach, et al., (1954) reported the isolation of H. capsulatum from the

atmosphere of a chicken house in which the organism had previously been recovered from the liter inside and soil outside. The digestive tract is also incriminated in transmission of H. capsulatum (Negroni, 1965; Gordon, 1965).

Cryptococcus neoformans is a spherical or oval encapsulated yeast, 4 to 7 microns in diameter averaging 10 microns with capsule, some-times reaching 18 microns (Littman and Zimmerman, 1956). Emmons, et al., (1963) reported that the organism may reach 20 microns in size. The size of the organism is dependent on the width of the capsule which may vary from practically non-existent to several times the thickness of the diameter of the cell itself (Laskowski, et al., 1960). Reproduction is by budding with single cells, occasionally by double budding. Identical morphology is expected at 25 C, at 37 C, and in tissue (Littman and Zimmerman, 1956).

Histoplasma capsulatum is a dimorphic fungus, exhibiting yeast-like characteristics in tissue and at 37 C on culture media and mycelial growth at 25 C (De Monbreun, 1934). At 25 C filamentous, branched, septate, hyalin hyphae, 2 to 5 microns in diameter are seen. According to Howell (1939), three types of conidia may be seen:

1) Large, spherical or pyriform bodies, 10 to 25 microns in diameter, covered by tubercules, 1 to 7 microns in length, separated from the inner protoplasm by means of a secondary wall.

2) Conidia similar to those described above, 5 to 20 microns in diameter, spherical or oval, with thin walls, commonly found on short pedicles on submerged mycelia.

3) Small conidia with thin walls, spherical or pyriform, 2 to 6 microns in diameter, sessile or on short pedicles found on submerged mycelia.

In tissue and at 37 C in vitro, Emmons, et al., (1963) described the organism as a typical oval budding yeast, 2 to 3 microns in width by 3 to 4 microns in length. Reproduction is accomplished by budding at the smaller end of the cell. The bud arises as a protrusion 0.2 to 0.3 microns in diameter. The attachment between mother and daughter cells remains narrow.

Smith and Jones (1966) stated that the gross lesions of crypto-coccosis are not diagnostic. However, they considered the microscopic lesions as diagnostic. Sections stained with hematoxylin and eosin show the cell wall and sometimes its contents, but the capsule remains unstained. The capsule can be stained by the mucicarmine technique and the periodic acid-Schiff method for glycogen.

The inflammatory response is essentially cellular. Tissue macrophages are the principle cellular elements observed and frequently the only inflammatory cells present. At times, there are many giant cells and dense infiltration by lymphocytes and plasma cells (Littman and Zimmerman, 1956).

Isolations of C. neoformans are accomplished from soil and tissues. When Emmons reported the first isolation from the soil, white Swiss mice were injected intrapertioneally with sample suspensions. After 4 to 6 weeks, the mice were killed and cultures were made from portions of the spleen and liver on modified Sabouraud's agar (Emmons, 1951).

Shields and Ajello (1966) described a medium (Diphenyl Agar) which provided for the selective isolation of C. neoformans from the soil. Botard and Kelley[1] described a medium (Modified Littman Oxgall Agar) which was excellent for isolation of the organism from contaminated dust samples. Laskowski, et al., (1960) stated that C. neoformans grew well on most non-inhibitory media. However, it would not grow on media containing cycloheximide.[2] Cultures should be incubated at 37 C and at 25 C. Good colony growth must be observed at 37 C for the organism to be C. neoformans (Emmons, et al., 1963).

Olander, et al., (1963) reported the presence of cells typical of C. neoformans in smears of mouse brain tissue stained with India ink as short as 7 days (I.V. injection) and as long as 5 weeks (intrapertioneal injection) after inoculation.

Pulmonary lesions of histoplasmosis are detected by X-ray examination. However, this examination is not diagnostic, as lesions of tuberculosis are similar. Like cryptococcosis, histoplasmosis is best diagnosed microscopically. The dominant feature in tissue changes is the extensive proliferation of reticulo-endothelial cells, many of which contain yeast forms of the causative organism, either a few, or so many that the cytoplasm is distended and tremendously enlarged (Smith and Jones, 1966).

Smith and Jones (1966) stated that H. capsulatum is best identified in tissues by staining with the periodic acid-Schiff, Bauer's

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[1]Submitted for publication

[2]Acti-dione, The Upjohn Company, Kalamazoo, Michigan.

or Gridley's method. Laskowski, et al., (1960) recommended the use of either Wright's or Giemsa's stain for identification in tissues.

Emmons in 1949 isolated H. capsulatum for the first time from soil. His recovery was accomplished by injecting mice intraperitoneally with soil sample suspensions. Approximately 3 to 5 weeks after inoculation, the mice were killed and portions of the liver and spleen were placed on Sabouraud agar. Two samples of 387 were positive for H. capsulatum.

Histoplasma capsulatum grows quite well on media currently being employed by many laboratories (Conant, et al., 1954; Beneke, 1957; Haley, 1964). H. capsulatum isolations should not be attempted on media containing cycloheximide if incubated at 37 C, as no growth or very poor growth is obtained. If cycloheximide is necessary for suppression of saprophytic fungi, cultures should be incubated at 25 C only (McDonough, et al., 1960).

MATERIALS AND METHODS

Sample Collections

Dust samples were collected at the Refrigeration Branch, Electrical Division, Physical Plant, Kansas State University from April 5, 1967 through August 29, 1967. Forty-two window unit air-conditioners received at the Refrigeration Branch for repairs were sampled. Selection of units to be examined were not qualified and all units were sampled.

Two dust samples were collected from each air-conditioner examined. One sample was obtained from the filter which filters air from the room cooled by the unit and the other sample was taken from the rear of the unit located outside the window and exposed to the atmosphere.

The collecting apparatus (Fig. 1) employed the Sentry Portable Air Sampler[3] as a vacuum pump on 2- 1000 ml Erlenmeyer flasks as water baths.

Fig. 1 - Photograph of collecting apparatus.
Vacuum pump (A) and water baths (B & C)

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[3]Model 25121, Gelman Instrument Company, Ann Arbor, Michigan

For the first 9 air-conditioners examined, each flask contained 250 ml. of sterile water as a filter. The remaining samples employed sterile physiological saline solution. All rubber and glass tubing was sterile prior to each examination.

After each sample was taken, the flask was restoppered prior to returning to the laboratory. Only the contents of the first flask (C, Fig. 1) were examined. The second flask (B, Fig. 1) served only as a filter for the vacuum pump.

The following information was obtained on each air-conditioner examined:

1. Location in room
2. Number of air-conditioners in room
3. Distance of air-conditioners from ground
4. Outside environment
5. Type of filter
    a. Permanent
    b. Washable
6. Time since filter was last changed or cleaned.

Mycology

At the laboratory, the sample and solution were filtered using a 3 micron, white, plain, 47 mm filter[4]. The filter paper and the residue remaining were placed in a sterile 25 x 150 mm test tube with screw cap. After weighing, 5 ml of sterile physiological saline

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[4] Millipore Filter Corporation, Bedford, Massachusetts 01730.

solution containing 1500 units of penicillin and 1.0 mgm of strepto-
mycin per milliliter were added to each gram of sample. The sample
was shaken for 2 minutes with a mechanical shaker[5] and allowed to
stand for one hour.

After one hour, 8 ml of the supernatant were transferred to a
sterile 12 x 100 mm test tube with screw cap. The sample was centri-
fuged[6] for 5 minutes at 2000 rpm's. The supernatant was removed with
a Pasteur pipette and the remaining residue was resuspended with 4 ml
of sterile physiological saline solution.

Following resuspension, 0.5 ml of the sample was injected intra-
pertioneally into each of four white Swiss mice and 0.1 ml was inocu-
lated and spread onto each of four plates of Brain Heart Infusion
agar with Chloramphenicol[7], 0.05 mg/ml, and Cycloheximide, 0.5 mg/ml,
four plates of Sabouraud Dextrose agar with Chloramphenicol, 0.05 mg/ml
and Cycloheximide, 0.5 mg/ml, and four plates of Diphenyl agar
(glucose, 10.0 g; creatine, 780 mg; chloramphenicol, 50 mg; diphenyl,
100 mg; Guizotia abyssinica extract, 200 ml; distilled water, 800 ml;
and agar, 20 g). The eighteenth through the twenty-first air-
conditioner samples were inoculated and spread on 4 plates of Littman
Oxgall agar. From the twenty-second sample until the conclusion,
Littman Oxgall agar plates were replaced with 4 plates of modified
Littman Oxgall agar (200 ml Guizotia abyssinica extract replaced
equal portion of diluent in production of Littman Oxgall agar).

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[5]Bimer and Amend, Fischer Scientific, Pittsburgh, Pennsylvania
15219.

[6]Size 1, Type SB, International Equipment Company, Boston, Mass.

[7]Chloromycetin, Parke, David and Co., Detroit, Michigan.

The mice were kept 8 to a cage for 28 days or until death; therefore, each cage maintained mice from 2 samples or one air-conditioner sampled. Identification of mice as to sample was maintained by notching the right ear of one group of 4.

The culture plates were split in groups of 2 and maintained at 37 C and at 25 C. Each plate was examined daily and results recorded at 7 day intervals for 28 days.

Suspect yeasts obtained from culture plates were stained with Gram's stain and observed under the microscope. Organisms which resembled C. neoformans were inoculated onto urea agar, potassium nitrate agar, four carbohydrates--dextrose, sucrose, maltose, lactose--and corn meal agar. Suspect mycelial growths from culture plates were stained with lactophenol cotton blue. After microscopic examination for the presence of characteristic conidia, all suspects of H. capsulatum were placed on Sabouraud Dextrose agar slants at 25 C and allowed to grow for 4 weeks. Weekly examinations were accomplished under the microscope after staining with lactophenol cotton blue. Yeast growth which resembled H. capsulatum were inoculated on Sabouraud Dextrose agar slants and incubated at 25 C. After inoculation, slants were handled in the same manner as suspect mycelial growth.

At four weeks, the mice were euthanatized with ether. Mice which died before 28 days were placed in a freezer (-15 C) until the end of the 28 days so that all mice in one test could be examined at the same time. The mice were dipped in Amphyl[8] and the abdomen opened using

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[8]Lehn and Fink Products Corporation, Toledo, Ohio 43612.

sterile equipment. Portions of liver and spleen were placed in
sterile tissue grinders[9] containing 1.0 ml of sterile physiological
saline solution. One-tenth milliliter of the tissue suspension was
inoculated onto each of four slants of Brain Heart Infusion agar and
four slants of Sabouraud Dextrose agar. Two slants of each were
incubated at 37 C and at 25 C. Cultures were examined weekly for
4 weeks and suspicious growth handled in the same manner described
for culture plates.

Microscopic Examination of Mouse Tissues

At necropsy, specimens of liver, spleen, left kidney and left
adrenal were placed in a solution of buffered 10% aqueous formalin
for fixation. The tissues were stained with hematoxylin and eosin,
the periodic acid-Schiff method and the Giemsa stain. The cranial
cavity was opened with sterile equipment and a small portion of brain
tissue was stained with India ink. Examination of India ink stained
tissues was accomplished within 30 minutes after staining.

Controls

Control samples were examined after every fourteenth air-conditioner
sampled. Using the same collecting apparatus previously described
for sample collections, a dust sample was obtained from an air-
conditioner (not included in this survey) located at the Veterinary
Research Laboratory, College of Veterinary Medicine, Kansas State
University.

9Scientific Products, Evanston, Illinois 60201.

Each control sample of dust taken from the air-conditioner was sterilized by autoclaving at 121 C for 30 minutes. After sterilization, the sample was divided into 2 portions. One portion was inoculated with C. neoformans[10] and H. capsulatum[11], yeast and mycelial phase of the organism. The other portion was handled in its sterile condition. The two portions were then run as a negative and a positive sample in the same manner described for an unknown dust sample. Positive identification of each isolation of H. capsulatum and C. neoformans was accomplished in the same manner as described previously.

[10]Mycology Laboratory, College of Veterinary Medicine, Manhattan, Kansas 66502.

[11]Ibid.

RESULTS

Sample Collections

Eighty-four dust samples were taken from 42 window unit air-
conditioners received for repairs from 17 buildings on the Kansas
State University campus, Manhattan, Kansas. Units were positioned in
the buildings anywhere from ground level to approximately 40 feet
above the ground. Thirty-four of the units examined were the only
air-conditioners in the room. Two rooms contained 2 units each and
both units were examined. Four units came from rooms with more than
1 unit, but the other units were not sampled.

The environments of the air-conditioners were determined to be
clear, dusty, bird feces present, dusty and bird feces present, and
trees and tree leaves present. The number in each environment is
given in Table 1.

Table 1 - Environments of Air-Conditioners

Environment	Number of Units
Clear	18
Dusty	6
Bird feces present	10
Dusty and bird feces present	6
Trees and tree leaves present	2

Thirty-nine air-conditioners examined employed the use of washable filters and only 3 units required the use of permanent filters which must be replaced when cleaned. The 39 reusable filters had been cleaned anywhere from one month to longer than 6 months prior to extraction of samples. The 3 permanent filters had all been in use approximately 6 months since last being changed.

Mycology

Cryptococcus neoformans was isolated from 1 of 84 dust samples taken. H. capsulatum was not recovered from any of the samples.

The one isolate of C. neoformans failed to grow on either the Brain Heart Infusion agar with antibiotics or Sabouraud Dextrose agar with antibiotics at 37 C or at 25 C. Brown coloring and good colony growth of the organisms were noted on both Diphenyl agar and modified Littman-Oxgall agar at 37 C and at 25 C. The colony appearance and microscopic morphology were characteristic of C. neoformans. Other laboratory results included: positive hydrolysis of urea agar; negative assimilation of potassium-nitrate agar; positive assimilation of dextrose, sucrose, maltose, but negative assimilation of lactose; and no pseudomycelium or chlamydospores formed on corn meal agar.

Four white Swiss mice inoculated with 0.5 ml of the sample died 5, 21, 22, and 25 days after being inoculated.

Only 3 of the 4 mice were used for more tests. The fourth mouse which died on the fifth day following inoculation was not used because it was too decomposed. C. neoformans was isolated from the tissues of

all 3 mice on Brain-Heart Infusion agar and Sabouraud Dextrose agar
at 37 C and at 25 C. Colony growth and microscopic morphology were
characteristic of C. neoformans.

Microscopic Examination of Mouse Tissues

Histoplasma capsulatum was not observed on microscopic examina-
tion of tissues from mice inoculated with portions of dust samples.
C. neoformans was observed in 4 tissues (liver, spleen, kidney, and
adrenal gland) stained with hematoxylin and eosin, periodic acid-
Schiff, and Giemsa stain of 3 mice inoculated with a dust sample pre-
viously described. Brain smears stained with India ink of the 3 mice
allowed for the organism to be seen with its characteristic large
capsule.

Controls

Control samples, autoclaved for use as negative controls, were
negative on all media, and animal inoculations proved to be the same.
Control samples inoculated with H. capsulatum and C. neoformans for
positive controls, yielded good results. The results obtained from
direct culture are recorded in Table 2, and the results obtained from
animal inoculations in Table 3.

Table 2 - Direct Cultures of Positive Control Samples

Test	Brain-Heart Infusion Agar with Antibiotics				Sabouraud Dextrose Agar with Antibiotics				Diphenyl Agar				Modified Littman Oxgall Agar*			
	H. capsulatum		C. neoformans		H. capsulatum		C. neoformans		H. capsulatum		C. neoformans		H. capsulatum		C. neoformans	
	37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C
Test 1	+	-	-	-	+	+	-	-	-	-	+	+				
Test 2	+	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+
Test 3	+	+	-	-	+	+	-	-	-	-	-	+	-	+	+	+

* Modified Littman-Oxgall Agar not used on Test 1.

Table 3 - Mouse Inoculation of Positive Control Samples

Sample	Mouse	Microscopic Examination of Tissues			Cultures of Mouse Liver and Spleen							
		India-ink Stain of Brain Smear	H & E, PAS, Giemsa Stains of Liver, Spleen, Kidney, Adrenal		Brain-Heart Infusion Agar				Sabouraud-Dextrose Agar			
		C. neoformans	H. capsulatum	C. neoformans	H. capsulatum		C. neoformans		H. capsulatum		C. neoformans	
					37 C	25 C	37 C	25 C	37 C	25 C	37 C	25 C
1	1	+	+	+	+	-	+	-	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+
	3	+	-	+	+	-	+	+	-	+	+	+
	4	+	+	+	+	+	+	+	-	+	+	+
2	1	+	+	+	+	-	+	+	+	-	+	+
	2	+	+	+	-	+	-	-	+	-	+	+
	3	-	+	-	+	+	-	-	+	+	-	-
	4	+	+	+	+	+	-	+	-	+	+	+
3	1	+	+	+	+	+	+	-	+	-	+	+
	2	+	-	+	-	+	-	-	+	+	+	+
	3	+	+	+	+	+	-	-	-	-	+	+
	4	+	+	+	+	+	-	+	+	+	-	+

DISCUSSION

This survey was conducted to determine the possible contamination of window unit air-conditioners with H. capsulatum and C. neoformans. Isolations were attempted from dust samples taken from two areas in each unit examined, one from the filter and the second from the rear of the air-conditioner which is positioned outside of the building. Eighty-four samples were obtained and examined from 42 air-conditioners located on the Kansas State University campus, Manhattan, Kansas.

Samples were taken from units positioned from ground level to 40 feet above the ground. The dust and debris in the air-conditioners varied from large amounts at the lower levels to sparse amounts at the higher levels. The availability of bird feces present was not constant at any height. Bird feces is the term used because no data was recorded to determine the origin of the fecal matter. Of the 42 air-conditioners examined, only 16 units or 38.1% contained bird feces.

Isolation attempts were made using conventional mycological procedures. However, attempts were also made to isolate H. capsulatum by direct cultures on media containing cycloheximide. Preliminary work with dust samples indicated that isolation of any one organism in pure culture was impossible due to overgrowth by saprophytic fungi. McDonough, et al. (1960), using H. capsulatum, indicated that the organism would grow only at 25 C when cycloheximide was employed in culture media. Laskowski, et al., (1960) did not indicate H. capsulatum to have any sensitivity to cycloheximide at any temperature and recommended its use for isolation of the pathogenic fungi. Since the

inoculation of mice was used, direct cultures from media containing
cycloheximide were attempted for H. capsulatum at 37 C and at 25 C.

This survey failed to yield any H. capsulatum isolates.
Manhattan, Kansas is on the western edge of an endemic area and fail-
ure to isolate the organism is not uncommon.[12] One isolate of
C. neoformans was recovered.

The air-conditioner (Fig. 2) yielding the isolate of C. neoformans
was approximately one foot above the ground. At the time the sample
was taken, the building surrounding the unit was ivy-covered with
the ivy in full growth. While obtaining the sample, the unit was
noticed to contain considerable amounts of dust, bird feathers, and
bird feces. The isolate was obtained from the dust sample taken from
the rear of the air-conditioner.

Fig. 2 - Photograph of contaminated
air-conditioner and surrounding building

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[12]L. Ajello, Communicable Disease Center, U. S. Public Health
Service, Atlanta, Georgia 30333, personal communication, 1966.

CONCLUSIONS

Although only one isolation of $\underline{C}$. neoformans was accomplished, its significance is not small. The presence in an air-conditioner of a pathogenic organism of both man and animals is of great concern.

The isolation was made in the rear of an air-conditioner. No isolations were obtained during this survey from the filter. Now that we know that it is possible for $\underline{C}$. neoformans to be found in an air-conditioner, a controlled study should be accomplished to determine if a unit can actually be a spreader of the organism.

The lack of isolating $\underline{H}$. capsulatum in this study is not discouraging. The area in which this survey was conducted is on the perimeter of an endemic area, and this may have been the reason for its not being isolated. In an endemic area, the air-conditioner could still be a very important spreader of the organism.

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A SURVEY TO DETERMINE THE OCCURRENCE OF HISTOPLASMA
CAPSULATUM AND CRYPTOCOCCUS NEOFORMANS IN AIR-CONDITIONERS

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AN ABSTRACT OF A MASTER'S THESIS

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Histoplasma capsulatum and Cryptococcus neoformans are recovered
from soil laden with pigeon, starling and chicken feces. The mode of
transmission of both organisms is by spore-laden dust. Due to the
association of pigeons, starlings, and other birds with window air-
conditioners, a survey of 84 dust samples from 42 units was conducted
at Kansas State University, Manhattan, Kansas. Isolations of
Histoplasma capsulatum and Cryptococcus neoformans were attempted.

Direct cultures were attempted on Brain-Heart Infusion agar with
antibiotics, Sabouraud Dextrose agar with antibiotics, Diphenyl agar,
Littman-Oxgall agar and Modified Littman Oxgall agar at 37 C and 25 C.
Mouse inoculations were made. After 4 weeks, mice were destroyed and
portions of liver and spleen cultured on Brain-Heart Infusion agar and
Sabouraud Dextrose agar slants at 37 C and 25 C. Sections of liver,
spleen, adrenal gland and kidney from mice were stained and viewed
under the microscope. Positive and negative control animals were han-
dled in the same manner described for samples.

Histoplasma capsulatum was not recovered in this survey. One
isolate of Cryptococcus neoformans was obtained. The contaminated
air-conditioner yielding the organism contained considerable amounts
of bird feces, bird feathers, and dust. The air-conditioner was lo-
cated approximately one foot above the ground in the window of an
ivy-covered building.