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Some Effects of Antibiotics on a Candida Albicans Population in the Intestinal Tracts of Chickens

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SOME EFFECTS OF ANTIBIOTICS ON A CANDIDA ALBICANS POPULATION IN THE INTESTINAL TRACTS OF CHICKENS

BY

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The poultry industry has been rapidly assuming a role of increasing economic importance during the last few years. Poultrymen are becoming extremely conscious of any disease which results in a decrease in production. Some of these diseases are the result of new intensive methods of management which place considerable stress on the individual bird. Certain pathogenic microorganisms are afforded an increased opportunity to overcome theprotective mechanisms of the chicken's body. A group of these opportunistic pathogens are the fungi capable of infecting animals. Their slight invasive power and dwelling often as commensals on the host animal has made the study and diagnosis of these organisms very difficult.

Probably the most important fungal diseases of poultry are aspergillosis, favus and moniliasis. Aspergillosis, an infection of the respiratory tract, is generally thought to be caused by Aspergillis fumigatus Fresnius. Favus, a chronic dermatomycosis is caused by Achorion gallinae. Moniliasis an infection of the mucous membranes lining the upper digestive tract is associated with Candida albicans and other members of the genus Candida. Of these three mycotic infections, only moniliasis has been more than occasionally implicated in diseases of epidemic proportions. (8)

An outbreak of moniliasis was observed by Jungherr (24) in a commercial chick hatchery where 10,000 of a total of 50,000 chicks hatched during a season, succumbed to this malady. Epidemics occuring in turkeys were reported from California by Gierke (16)

and Hinshaw (21). Jordan (23) and Buxton et al (9) observed this disease in the British Isles while Hart (18) reported several outbreaks in turkeys during the dry season in Australia. McGaughey's work, as described by Chute and O'Meara (10), found moniliasis to be prevelant in various domestic fowl on the island of Ceylon.

Moniliasis is generally thought to be associated with unsanitary conditions and possibly secondary to certain debilitating factors (6, 21, 23, 24). It is also assumed that that Candida albicans organism present as a commensal or parasite in some intestinal tracts, may serve as a reservoir of infection (7).

It was the purpose of this study to determine the effects of certain antibiotics in the form of feed supplements on this intestinal parasite, and to learn something of the cultural characteristics of this yeastlike fungus.

LITERATURE REVIEW

One of the major difficulties in studying Candida albicans and the other mycelia producing non-ascospora forming yeastlike fungi was the inability of the early investigators to agree upon what criteria ought to be used in the classification of these organisms. As a result, many synonyms are to be found in the literature dealing with the yeastlike fungi. Conant (11) reported that he had found a total of 172 synonyms for the organism that is now known as Candida albicans. He compiled a list of the more important names which have been used down through the years. This collection of synonyms included Oidium albicans Robin, 1853; Monilia albicans Zopf, 1890; Endomyces albicans Vuillemin, 1898; Monilia Pinoyi[1] Castellani and Chalmers, 1913; Monilia psilosis Acford, 1917; Parasaccharomyces Asfordi Anderson, 1917; Monilia castellanensis Castellani and Chalmers, 1920; Monilia richmondi Shaw, 1926; Monilia Aldoi Pereira, 1917; Mycotoruloides tradis Longeron and Talice, 1932; Syringospora insmerabilis Dodge, 1935 and Candida albicans Berkhout, 1939.

Rearici (20) classified the microorganism Candida albicans according to the following taxonomical scheme:

Class:	Fungi Imperfecti
Order:	Moniliales
Family:	Torulospsidaceae
Tribe:	Candidoideae
Genus:	Candida
Specie:	albicans

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[1]International Rules of Botanical Nomenclature recommend that specific names derived from personal or generic names begin with a capital letter.

Langenbeck (1839), as described by Skinner (35), was the first to report this organism which he found growing in patches in the oral cavities and digestive tracts of people who had succumbed to typhoid fever. Robin (1853), also described by Skinner (35), isolated a similiar organism from a case of thrush and gave it the name Oidium albicans. Quinquad, a reported by Skinner, (35) recognized the fact that this fungus did not belong in this genus and placed it in a new one called Syringospora. The drawings and descriptions produced by him are definite enough to ensure that this organisms, which he called Syringospora Robinii is the same one that is presently referred to as Candida albicans.

Zopf (1890), as reported by Conant (11), gave this organism the generic name of Monilia which has been one of the more commonly used names in spite of the fact that it is invalid because it had been previously given to a group of ascospore forming fruit pathogens by Persoon.

Benham (2), in what is now considered a classical paper by microbiologists, provided the first practical basis on which the non-ascosporous Monilias could be identified and classified. She combined a study of both physiological and morphological properties and found that neither set of characteristics was sufficient when used alone. All pathogenic Monilias were believed by her to belong to the same species and any variation among strains was of no consequence.

Stovall (36), in a taxonomical study of the Monilias suggested a set of environmental conditions under which biological characteristics

could be kept relatively constant and could be readily demonstrated.

Martin (29) and associates produced the first clear cut scheme for classification of the Monilias of medical importance. They combined colony growth characteristics on Sabouraud's, blood and serum meal extract agars with growth in Sabouraud's broth and one per cent glucose, maltose, sucrose and lactose sugar solutions. Their scheme recognises one pathogenic species which is albicans and six nonpathogenic species which are tropicalis, pseudotropicalis, Krusei, parakrusei, stellatoidea, and Guillermondi. This method of classification and differentiation has been widely accepted by microbiologists.

The genus name Candida was chosen by a group of interested workers at an informal meeting held at the Third International Microbiological congress in 1939. This name had been suggested earlier by Berkhout in 1929 but not accepted as it was thought that the perfect stage of reproduction of this group would eventually be discovered. Until that time the generic name Monilia was considered sufficient. (35) The Candida name has been gradually accepted by most taxonomists. A notable exception is Dodge (14) who chose to retain the original valid name Syringospora. His equivalent for the species name albicans is exorabilis.

Wickerham and Rettger (43) in a taxonomical study of Monilia species from various sources concluded that the strains isolated from chickens and turkeys were identical to their isolates from human sources.

An infection of chickens by Candida albicans and other members of the genus Candida has been referred to by the following names:

stomatitis, oidica, muguet, soor, oidiomycosis, sour crop and moniliasis. This mycosis is primarily an infection of the upper digestive tract with the crop being the focal point of infection.

Elbert, as cited by Bullis (8), reported a case of thrush in which the upper digestive tracts of affected chickens were ulcerated and scaly. Schlegel, also reported by Bullis (8), noted that the proventriculus was the principle organ involved with the crop, mouth and pharynx being implicated in some cases. Both of these early workers isolated the organism Oidium (Candida) albicans from the lesions which were present.

Jungherr (24) was the first to report of moniliasis manifesting itself in epidemic proportions among chickens. The diseased condition was characterized by whitish ulcers or pseudomembranes in the crop, brownish or mucoid deposits in the proventriculus and ulcers in the gizzard. Lesions in small chicks were easily missed as they were often very small. The predominating organism which was isolated from the diseased organs was a yeastlike fungus which resembled Monilia (Candida) albicans: the other types resembled Oidium lactis and Monilia (Candida) krusei. They were isolated from the intestines, gall bladder and the liver in addition to the visibly affected organs. He interpreted the presence of the organisms in the liver and gall bladder as an indication of septicemia. Slight pathological changes, with focal necrosis was considered as an indication of toxin production. The disease was reproduced by feeding fecal materials from infected birds and pure cultures of Monilia (Candida) albicans with deaths occuring

as early as 10 days after inoculation. Eggs from an infected flock when hatched in a sterilized incubator, produced chicks which were infected with Monilia (Candida) albicans.

Blaxland (5, 6) described an epidemic of moniliasis of turkeys in England as similier to that described by Hinshaw (21) in California. Lesions, if present, were generally confined to the crop and appeared as flocculent greyish white exudate slightly adherent to the underlying membrane in acute cases. In chronic cases, he observed the membrane to be thickened and coarse. He observed poults dying from acute cases to be normal appearing and to possess no diagnostic symptoms while those that died during chronic outbreaks lost weight and became generally unthrifty. He was unable to reproduce the disease experimentally except by placing normal birds with diseased ones. He could not prove that debilitating conditions were a cause of this affliction in spite of the fact that various debilitating factors could be observed in many outbreaks. No evidence of egg transmission of Candida albicans as reported by Jungherr (24) was found. In later experiments he was able to reproduce the disease by the injection of the scrappings from infected crops. He believed that crop lesions were not directly connected with the disease as he observed them in normal appearing birds with no signs of clinical disease (7).

Candida albicans has been reported to increase in incidence as a result of oral therapy with various antibiotics by Harris (17), 1950; Woods, et al (44), 1951; and Lipnik, et al (27), 1952. Huppert (22) reported that by orally administering aqueous solution of Aureomycin, Chloromycetin, dihydrostreptomycin, Magnamycin, Neomycin, Terramycin, Erythromycin, penicillin and tetracyclins, mice were predisposed

toward the establishment of an experimental Candida albicans population in their intestinal tracts.

Sieburth (34) used therapuetic levels of Aureomycin and Terramycin (1,000 PPM) in an ordinary chick ration to establish Candida albicans in the intestinal tracts of young chicks and poults. The only yeastlike organism which appeared, voluntarily, with the feeding of these two antibiotics was Torulopsis (Cryptococcus) holmbianus. Candida albicans appeared in the feces of these birds only after it was orally injected into the crops of the test birds. He found that the birds fed Terramycin showed detectable numbers of microorganisms which were antagonistic toward Candida albicans. These organisms were identified as strains of Proteus mirabilis. Birds fed the antibiotics showed a larger yeast count and more extensive crop infection than the control birds. However, the groups fed no antibiotics were more emaciated and all died within 15 days. No apparent difference was detected between the yeast populations of the Aureomycin and the Terramycin fed groups.

Considerable interest has been shown by several investigators in the effects which Candida albicans might have on the chick embryo. Moore (31) in an in vivo study of 15 different fungi including Candida albicans, which produced various kinds of lesions in man, showed that all of the fungi could be cultivated on the chorioallantoic membrane of 10-14 days old embryos. He observed luxuriant growth as early as five days after inoculation.

Meyer showed that Candida albicans and Candida stellatoidea could kill 10 day old embryos in 48 hours with severe lesions appearing

in less than 24 hours (30). With the exception of Tropicalis which produced mild lesions, the rest of the Candida species were not pathogenic for the embryo.

Morris (33) inoculated 11 day old embroys intravenously, with Candida albicans cells to study the effect of this yeastlike fungus on the tissues. He observed resulting lesions to be confined to the chorioallantoic membrane, and to resemble those of mucous membranes. Embryos, not quickly overcome, developed focal areas of liquefaction necrosis which were associated with hemorrhages.

Foley (15) injected 500 Oxford units of Penicillin along with Candida albicans and Candida albicans, var. stellatoidea cells into 10 day old embryos to show that this antibiotic could increase the pathogenicity of these organisms. Treated embryos succumbed as early as 2 days while untreated ones lived at least five days. When traumatic deaths were taken into account, no significant difference could be detected between the number of deaths in the two groups. He was able to show the same enhancing effect in rabbits inoculated intravenously. Fewer kidney lesions were noted in the rabbits which received no penicillin as compared to the treated group which received daily doses of 150,000 units of penicillin and died in four days.

Aaronson (1) reported that heat killed Candida albicans cells had no visible effect on the morphology of trypsinized chick embryo cells, nor did live cells when added to the tissue culture medium in concentrations less than 100,000 cells per tissue culture unit. He found that oxytetracycline hydrochloride caused the death of the trypsinized cells within 48 hours in concentrations of 0.1 milligram

per milliliter of tissue.

Moniliasis in chickens and other domestic fowl is commonly diagnosed by the observation of characteristic proliferative, relatively non inflamatory lesions in the upper digestive tract. Heavy growth of yeastlike organisms from artifically cultured materials from the intestinal tract is also observed (8). Underwood (40) perfected a method of detecting crop lesions by using a McCarthy forblique panendoscope which was inserted into the crop via the mouth of the bird. He noted that infected crops showed severe corrugations, whitish streaks, erosions and diphtheritic formations. Some lesions were the size of a grain of wheat while others were diffused or too small to be easily observed. Experimentally infected crops showed only a mild diphtheritic membrane formation.

References in the literature pertaining to the treatment of Candida albicans infections in poultry are very limited in scope. Jungherr (25) reported that denatured alcohol and various coal tar disinfectants were not effective against this yeastlike organism. An iodine preparation was recommended as a disinfectant, and Epsom salts followed by copper sulfate in the drinking water as a treatment. Hinshaw (21) suggested a 1:2000 solution of copper sulfate in the drinking water as a treatment for turkeys.

Underwood et al (41), in a critical study of copper sulfate, found that it had no preventative or therapuetic effects against strains of Candida albicans which he had previously observed to be able to produce crop lesions in chickens and turkeys. In fact chickens which received the copper sulfate, appeared to have more extensively

infected crops. He was unable to increase the intensity of the lesions by injecting Terramycin and tetracycline or by scarifying the membraneous lining of the crop.

Gentian Violet, diluted 1:10,000 in 10 per cent alcohol, has been used against external lesions in human medication. Further dilution is necessary for internal use, but treatment must be limited to four or five days in order to avoid injury to the mucous membranes (11). Hesseltine (19) employed Lugol's iodine solution as a topical application to vaginal infections by Candida albicans. She also used autogenous vaccines in very resistant cases with good results. X-ray therapy has also been used by her to remove external lesions in the cases of human moniliasis.

Antibiotics have played a very small part in the treatment and prevention of infections by Candida albicans and other members of the genus Candida. The only antibiotic which has been shown to be somewhat effective is nystatin. This antifungal antibiotic is produced by certain strains of the organism Streptomyces noursei, and has been used to some extent in the treatment of human moniliasis (37). Only one report has been found where this antibiotic was used to combat Candida albicans infections in chickens. Yacowitz et al (45) was able to prevent the spread of what was termed a highly virulent strain of Candida albicans from a 100 per cent infected group of chickens to an adjoining uninfected group by feeding nystatin in a concentration of 50 grams per ton of feed.

PROCEDURES AND RESULTS

This investigation was initiated as an attempt to isolate the organism Candida albicans from the intestinal tract of a chicken by plating a measured amount of fecal material in a simple medium composed of one per cent peptone, four per cent maltose and one and one-half per cent agar. Bacterial growth was suppressed by a pH of 5.6 and the addition of 20 units of penicillin and 40 micrograms of dihydrostreptomycin per milliliter of culture medium.[2] This medium referred to as Sabouraud's Maltose agar (12) was obtained from the Difco Laboratories in a dehydrated form.

One colony appeared after 48 hours of incubation which bore the macroscopic characteristics of Candida albicans. This colony was creamy white and moist appearing with a relatively high pulvinate elevation possessing a faint yeasty odor suggestive of Saccharomyces cerevisiae. Microscopic examination of the edge of this colony with the low power objective, revealed the presence of clearly visible oval to spherical cells. Elongated cellular structures were observed which were thought to be rudimentary mycellium. (See plate I page 13)

Inoculum from the top and center of this colony was streaked onto nutrient agar to encourage the growth of any bacterial contaminants, and thus obtain this isolate in pure culture. This organism was then inoculated into glucose, maltose, sucrose and lactose broths. The

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[2] The penicillin and dihydrostreptomycin used in the laboratory phase of this study was donated by Beebe Laboratories, Inc., St. Paul, Minnesota.

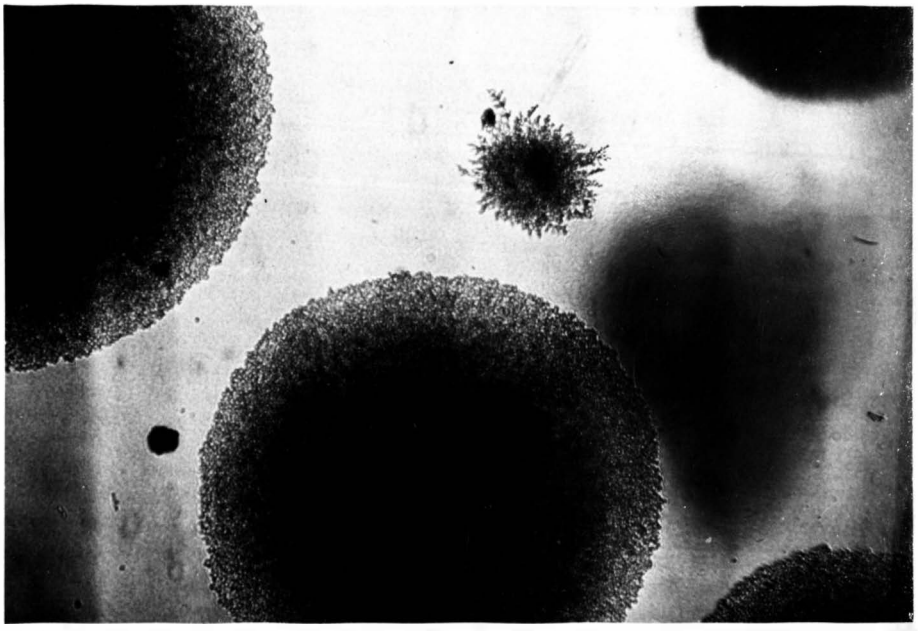

Plate I. Candida albicans colonies on Sabouraud's Maltose Agar in both the yeast and mycellial phases. (100X)

Plate II. A Candida albicans mycellium with terminal chlamydospores. (100X)

fermentation of the first three sugars, lack of turbidity in broth cultures and its colony characteristics fitted the identification scheme originated by Martin et al (29). What remained for positive identification of this isolate as Candida albicans was the demonstration of this isolates's ability to produce a thick walled, spherical body at the end of certain hyphe. This structure is an asexual spore and is commonly referred to as a terminal chlamydospore. (See plate II page 15).

A medium which is composed of water soluble carbohydrates other than reducing sugars, seems to stimulate Candida albicans to produce chlamydospores after the organism has formed mycellia (32).

In this attempt to prove or disprove this isolate's ability to sporulate, several media reported in the literature were compounded according to the particular author's recommendations. Tsachdjian's (39) white rice agar, Bacto Corn Meal Agar (13), Nickerson's (32) reducing sugar-free polysaccharide agar and Bernhardt's (4) yellow corn meal agar were prepared and dispensed into sterile petri dishes in 20 milliliter amounts. Two separate batches of corn meal agar were made, using two different brands of commercially prepared yellow corn meal.

The solidified media were inoculated by stabbing and streaking the surface of the agar with the organism. The sites of inoculation were covered with sterile glass cover slips and incubated for one week at room temperature. The plates were examined at 24 hour intervals with the low power lens of a microscope. Typical mycellial growth with an abundance of blastospores was observed after as early as 24

hours of incubation. These structures very closely resembled the camera lucida drawing of Banhem (2). However, no chlamydospores were observed in any of the cultures. That the unknown organism was one of the Saccharomyces species which possess similiar fermentative characteristics was ruled out because of the appearance of mycelial growth.

Continuing on the premise that this isolate was Candida albicans, it was decided to repeat these trials using the media which produced the best mycelial growth. The media of choice were the two batches of yellow corn meal agar made according to the method of Bernhardt (4). In this trial, it was decided to utilize some type of slide culture method in an attempt to improve visibility of the undisturbed cell structures. The first method tried was one described by Henrici (20) which consisted of covering a clean, sterile microscope slide with a thin layer of an appropriate medium, streaking this agar with the organism and covering the inoculum with a cover slip. This slide was then incubated in a sterile petri dish with a piece of moistened paper or cloth, to retard evaporation of the moisture from the medium. This method or some variation of it is widely used for cultivation of the filimentous fungi.

Subsequent usage of Henrici's (20) method revealed that moisture was lost from the agar too rapidly to allow for an incubation period of any length. Also, the development of mycelial growth seemed to be greatly impaired by any decrease in the moisture content of the corn meal agar. Visibility through this apparatus was excellent.

In an attempt to eliminate some of the cumbersomeness of the Henrici method and at the same time to try to retard the evaporation of

the medium, an apparatus was constructed by the author to more closely fit the needs of this moisture sensitive fungus. An ordinary one by three inch microscope depression slide was fastened to the bottom of a clean petri dish with plasticene taking care not to obstruct vision through the slide depression and the petri dish. The lid of the petri dish was then replaced and the entire dish was sterilized by autoclaving. A tiny bit of inoculum was then placed in the bottom of the slide depression and covered with cooled melted corn meal agar. Upon solidification of the agar, the dish was inverted and the cover was partly filled with water to form a moist, air tight chamber. Examination after less than 24 hours incubation at room temperature revealed the presence of a profuse extention of blastospore bearing mycelium from the edge of the mass of yeast cells which constituted the inoculum. Also present were a few very obvious structures which were identical to Benhams's (2) illustrations of terminal chlamydospores. (See plate II page 13). Further incubation revealed no additional chlamydospore production. By simply maintaining the water level in the petri dish under the culture, the medium sustaining the fungus was prevented from undergoing dehydration almost indefinitely.

Although both corn meal media produced abundant mycelial growth, only the medium made with Quaker's brand corn meal was able to cause the isolate to produce chlamydospores. The other commercial brand of corn meal was Argo.

This sporulation medium was prepared in both cases by heating six grams of yellow corn meal in 150 millilitere of tap water at 60 degrees centigrade for one hour. A water bath was used to prevent

scorching of the medium. A clear solution was easily obtained by centrifugation of the mixture at approximately 100 revolutions per minute and decanting off the centrifugate. All material causing turbidity was pelleted at the bottom of the centrifuge tube. After restoring the mixture to its original volume with tap water, one and one half grams of agar were added to the medium which was then autoclaved at 15 pounds of steam pressure for 15 minutes.

Benham (2) reported considerable variation in the ability of her strains of Monilia (Candida) albicans to ferment various carbohydrates. Avian source isolates studied by Jungherr (25) were observed by him to produce acid and gas from glucose, maltose, levulose and mannose, slight acid from sucrose and galactose and no change with dextrin, inulin, lactose and raffinose. Using methods and materials recommended by the MANUAL OF MICROBIOLOGICAL METHODS (28), the C-1 strain and nine other isolates of Candida albicans which fitted the identification scheme of Martin et al (29) were tested with these carbohydrates to determine if they were identical to those investigated by Jungherr. With the exception that two isolates showed slight acid in inulin and strong acid production in galactose, the strains as a whole were identical to those reported by Jungherr. It was also observed that gas production was not consistent in carbohydrate solutions of less than one per cent. Bacto Phenol Red Broth Base was the medium to which the carbohydrates were added.

These Candida albicans isolates were also observed to be unable to produce indole, acetylmethylcarbinol or hydrogen sulfide. Nitrates were not reduced after two weeks of incubation, nor was urea or starch

hydrolysed. These isolates were able to use ammonium sulfate as a source of nitrogen but were unable to use citrate as a source of carbon. Gelatin was not liquefied and an extremely alkaline reaction was observed in litmus milk. The only effect which these isolates seemed to have on rabbit erythrocytes was a slight browning of these cells in the vicinity of the colonies. A temperature of 37 degrees centigrade and a pH ranging from 5.5 to 7.0 appeared to produce the best growth of these Candida albicans isolates.

An antibiotic sensitivity screening test was set up to determine the effectiveness of several widely used antibiotics against these 10 isolates. Bacto Sensitivity Disks were asceptically dropped on the surface of nutrient agar plates which had been seeded with viable Candida albicans cells. No sensitivity was shown toward bacitracin, neomycin, oleandomycin, novobiocin, magnamycin, furadantin, tetracycline, oxytetracycline, chlortetracycline, erythromycin, polymyxin and chloromycetin. Only nystatin seemed to produce any in vitro inhibition.

Candida albicans has been reported by Blaxland et al (7) and Jungherr (23) to occur as a parasite or commensal in the intestinal tracts of some chickens and turkeys. Jordan et al (23) found a slightly greater incidence of this yeastlike organism in birds which were on an intensive system of production. As this organism was easy to isolate from hens being used by the South Dakota State College Poultry Department in experimental work, it was attempted to determine to what extent normal Candida albicans infection was influenced by various antibiotic feed supplements. Available for this study were four groups of hens

which were being used in an antibiotic supplementation and egg production experiment by the Poultry Department. These birds were fed a laying ration considered adequate in all nutritional requirements. They were divided into four groups, to which the following antibiotics were added in the ration: Group I, penicillin; Group II, penicillin and terramycin; Group III penicillin and nystatin and Group IV, all three antibiotics.

The number of viable Candida albicans cells present in the expelled feces, was used as an index of the extent of infestation of the intestinal tract. Fecal material was collected from each of six hens by placing clean paper under the cage of each bird. This material was transferred to sterile containers as soon as it was expelled by the hen. One gram aliquots were weighed out and ground with 99 milliliter of sterile buffered saline for five minutes using a Waring Blender with a small size grinder head. One milliliter amounts of this material were plated out in duplicate to constitute a dilution of 1:100. Sabouraud's Maltose agar containing 20 units of penicillin and 40 micrograms of streptomycin per milliliter of medium was used as the selective medium. The plates were incubated at 37 degrees centigrade for 48 hours before being examined for colonies which resembled Candida albicans.

A review of some pertainent literature revealed that some subsurface colonies of Candida albicans appear in dull colored, filimentous forms (12). These were also included in the colonies which were subcultured for positive identification.

In this study, a new selective medium called Page's-Levine Agar[3] was used to facilitate the identification of large numbers of yeastlike colonies. This medium contained a water soluble colorless tetrazolium chloride salt which can be reduced by some microorganisms to an insoluble formazan of various shades of pink or red. A neomycin complex was used to inhibit bacterial growth. Candida albicans, when streaked on slants of this medium, produced cream to pink colored growth (38). Bacteria able to grow are red in color, as are most yeast and molds. An exception is Candida krusei which produces a chalky white growth.

The results of this study are shown in Table I, page 30.

The only hens which showed sizable Candida albicans populations were those fed the diet supplemented with only Penicillin (Group I).

TABLE I. Candida albicans Colonies Isolated from Antibiotic Fed Laying Hens

	Group			
Hen	I	II	III	IV
1	10[a]	0	1	1
2	11	0	0	1
3	13	0	0	0
4	30	0	0	7
5	12	0	0	0
6	10	0	0	0

[a]The number of colonies per milliliter of one hundred fold diluted fecal material. This number is an average of two plates

[3]This medium was supplied in sterile sealed slants by E.R. Squibb and Sons, Inc. through the courtesy of Dr. Harold Yacowits, New Brunswick, New Jersey.

The other three groups which received nystatin in addition to penicillin (Group III) Terramycin in addition to penicillin (Group II) or both in the antibiotics in addition to penicillin (Group IV) showed infestations of a much lesser degree. These enumerations were repeated a few weeks later, and results were obtained which compared closely to the original counts.

The presence of nystatin was considered sufficient reason for the lower Candida albicans populations in groups III and IV. The antibiotic Terramycin considered ineffective against this organism, also showed a much smaller incidence of Candida albicans in the group of birds which received it as a supplement. It was thought that antagonistic microorganisms might be present in these birds that were fed a low level of Terramycin as was reported by Sieburth et al (34) to be true of chickens and turkeys fed very high levels of this antibiotic. It was also thought possible that these antagonists might be sensitive to penicillin in low levels, and this in addition to the stress incurred by intensive production is responsible for the relatively large Candida albicans incidence in the penicillin fed group.

The investigation to determine the presence or absence of this type of antagonistic organism was carried out by using a modified form of Fleming's technique (26) for the isolation of antibiotics producing microorganisms. Fecal material was collected on clean paper as in the preceeding study. Tenfold dilutions were made using sterile saline in a range of 10^{-2} to 10^{-8}. Nutrient agar was used as the growth of aerobic bacteria was desired. Wakeman (42) in a study of

antibiotic producing microorganisms, found that this growth inhibiting property was confined to the aerobic and facultative anaerobic microorganisms.

Ten hens were individually sampled in each of three groups which were utilized for this study. One group of chickens was fed a nutritionally adequate ration which was supplemented with Terramycin, a second group received a ration supplemented with penicillin while a third group was fed a ration which was devoid of any antibiotics. These hens were also from an antibiotic supplementation and egg production experiment being conducted by the Poultry Department at South Dakota State College.

Antagonistic organisms were detected by first plating out one milliliter amounts of each dilution with the afore mentioned medium and incubating these plates at 37 degrees centigrade for at least 48 hours. At the end of this period, the plates were overlayed with another layer of the same medium to which had been added viable Candida albicans cells in a concentration heavy enough to provide sufficient growth and insure zones of no growth with distinct edges. The proper concentration was determined earlier as one milliliter of a 24 hour glucose broth culture added to 100 milliliters of cooled but not yet solidified nutrient agar. After incubation at 37 degrees centigrade for 24 hours, the plates were examined for symetrical zones of no growth in the upper layer of the medium. (See plate III page 23).

Plates which contained these zones of no growth were retained for the location and identification of antagonistic colonies. The colony or colonies which appeared at the geometric center of a zone

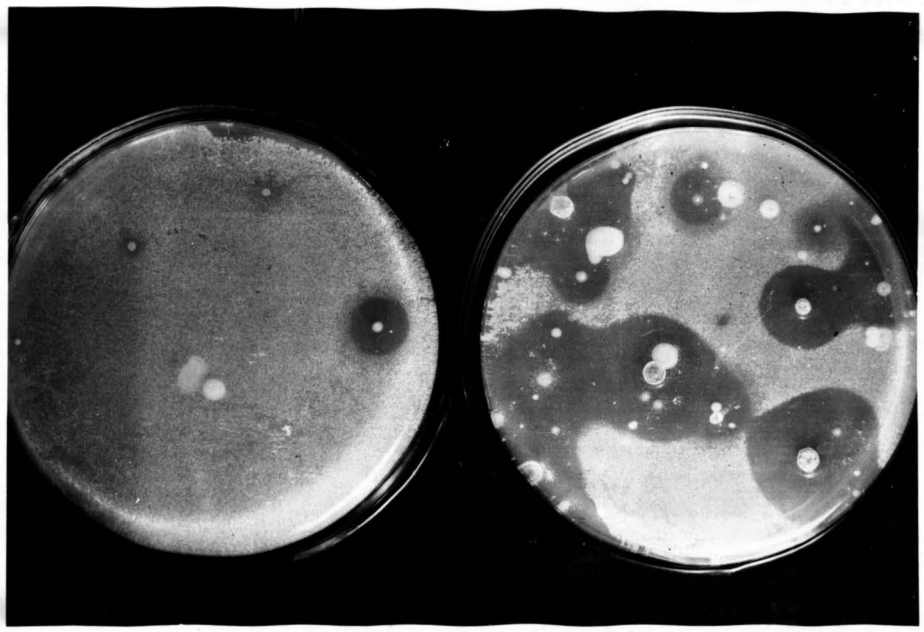

Plate III. Zones of inhibition caused by microbial antagonists in a medium inoculated with Candida albicans isolate C-1.

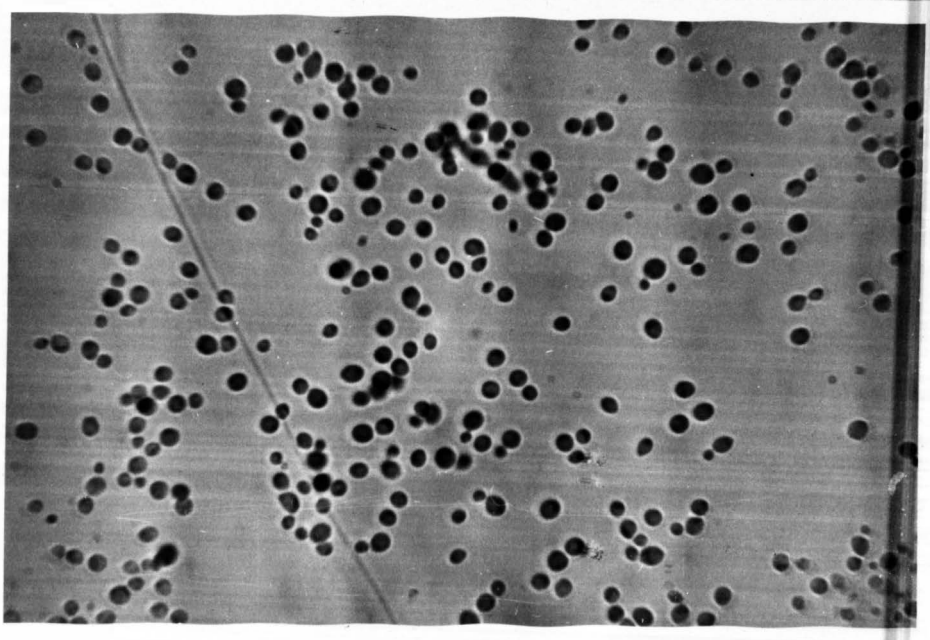

Plate IV. Candida albicans isolate C-1 cells from a 48 hour Sabouraud's Broth culture.

were subcultured onto nutrient agar slants and Kelner's method (26) of investigating large microbial populations for antibiotic activity was utilized to determine which colonies possessed this property. This method is essentially Fleming's method with a slight modification which consist of placing a layer of semi-solid agar containing adequately dispersed colonies between a foundation layer of sterile agar and an overlying layer of an appropriate medium containing the supposedly sensitive organism.

The results of this investigation are shown in table II.

TABLE II. Chickens Showing Evidence of Harboring Antagonists

Group	Diet	Hen									
		1	2	3	4	5	6	7	8	9	10
1	Basal only	-	-	-	+*	-	-	-	+	-	-
2	Terramycin	-	+	-	-	-	-	-	-	-	+
3	Penicillin	-	+	+	-	-	-	-	-	-	-

*Denotes the presence of microbial antagonists.

Only the presence of these organisms was recorded as the counts from the birds were fairly close. With the extreme variation in the diameter of the zones of inhibition (0.5 - 20 cm), it was believed that the number of such organisms present in each chicken was of no importance.

From the results recorded in table II, it appeared that the presence of penicillin and Terramycin in the rations of these hens had no effect on the number of birds exhibiting these organisms in their intestinal tracts.

Twenty of the antagonistic cultures were retained for identification purposes as it was desirable to know if any of the isolates were Proteus mirabilis which had been reported by Sieburth (34) to be antagonistic towards Candida albicans.

Using procedures and materials suggested in the Manual of Microbiological Methods (28) four of the isolates were identified as being identical to the criteria set down by Bergey's Manual of Determinative Bacteriology (3) for Micrococcus epidermidis. Five cultures were thought to belong to the genus Bacillus on the basis of staining and morphological characteristics. With the exception of two isolates which were lost, the rest of the antagonists were shown to be of the Streptomyces group. None of the latter two groups were identified as to the species to which they belonged. No organisms which resembled Proteus species were observed as being antagonistic toward Candida albicans.

Although it could not be determined why Terramycin failed to predispose the birds towards the establishment of a Candida albicans population as did penicillin, it was decided to investigate the effects of a low level antibiotic feed supplement on the establishment and build up of a Candida albicans population of both natural and experimental origins. That high concentrations of antibiotics comparable to therapeutic levels can result in the overgrowth of these yeastlike organisms in the intestinal tract is a fairly well established fact. However, no information seems to be available concerning low levels of orally administered antibiotics and their effect on this intestinal parasite.

An experiment was set up in which the establishment and build up of Candida albicans was observed and compared in three groups of birds which were given different levels of Aureomycin in the form of Aurofac-10*. Group 1 received Aureomycin at a level of 900 grams per ton while group 2 received 50 grams per ton and group 3 was fed a ration devoid of any antibiotic content.

The 33 Leghorn cockerels used in this experiment, when one week of age, were divided into three groups and housed in electrically heated batteries. Conditions of stress were kept at a minimum by ample cage space per bird, proper temperatures, clean water and a diet considered to be adequate in all nutritional requirements.

The three groups of birds were checked at five and six days after the start of the experiment for the presence of Candida albicans in their intestinal tracts by plating out composite samples of fecal material from each group. Sabouraud's Maltose agar was used in the manner described in preceeding experiments. The only microflora which could be cultured were a few fungi of the filimentous type. Samples of fecal material were collected on clean paper placed on the droppings tray under each battery. No less than 10 samples were pooled from each group to form the sample from that group. Plating of these samples in Sabouraud's Maltose agar revealed the presence of no detectable yeast or yeastlike populations in any of the three groups during the 12 day period. Filimentous fungi seemed to be more abundant on the plates from the two groups of cockerels fed the anti-

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*Aurofac-10 contains 10 grams of Aureomycin per pound of additive.

biotic supplemented rations.

As it appeared that under the conditions of this experiment, neither a high nor a low concentration of an antibiotic was able to induce a voluntary invasion of the intestinal tract by Candida albicans or any other yeast or yeastlike fungus, it was decided to experimentally infect the birds in each of the three groups with viable cells of one of the strains isolated from a hen in an earlier study. This was the strain designated C-1 which conformed to the criteria set down by Martin et al (29) for the identification of Candida albicans.

Before strain C-1 was inoculated into the chickens, it was checked for it's ability to bring about the condition referred to as moniliasis. Massive doses of cells (one milliliter) were injected into the crops of two cockerals which were one week of age. The injections were made on two successive days via the oral cavity and esophagus with a dulled 19 gauge needle and a two and one-half milliliter syringe. The two birds were observed for one week for any outward symptoms of the infection such as listlessness, rough plumage or death. None of these symptoms were observed although plating of fecal material from both of the birds in Sabouraud's Maltose agar revealed that Candida albicans was now present in each of the cockerals. These two birds were retained for a month, and then sacrificed in order to examine their upper digestive tracts for the presence of lesions considered to be typical of moniliasis.

No lesions were found in the mucous membranes lining the esophagus, crop or the proventriculus of either bird. Scrappings from the mucous membranes lining the crop were streaked onto Sabouraud's

Maltose agar plates and incubated at 37 degrees centigrade, for 48 hours. A heavy growth of yeastlike colonies was observed on each plate. Some of the colonies well separated from the other growth were subcultured in corn meal agar in the form of the slide cultures described in a previous experiment. All of these isolates produced chlamydospores, and thus were identified as Candida albicans. (See plate II page 13).

The inoculum to be used in this phase of the experiment, was prepared by removing the cells from a 48 hour Sabouraud's Dextrose Broth culture by centrifugation, and resuspending them in an equal volume of sterile, peptone buffered saline. (See plate IV page 23). The cells were administered by the use of a two and one-half milliliter syringe with a 19 gauge needle, two inches in length, and having a smooth blunt point. The needle was carefully inserted into the esophagus by way of the mouth, and 0.5 milliliter of the Candida albicans cell suspension was deposited deep in the esophagus of each bird.

Starting 24 hours after the third groups of cockerals had been inoculated, a composite sample of fecal material was collected from each group of birds in the manner described in the preceding experiment. These samples were diluted in sterile saline (one hundredfold) and plated in Sabouraud's Maltose Agar. Yeastlike colonies were identified by subculturing them onto Pagano-Levine agar slants (38).

The results of this experiment are enclosed in Table III page 29.

TABLE III. CANDIDA ALBICANS RECOVERED FROM INOCULATED CHICKENS

Group	Antibiotic Content (gms./ton)	Days					
		0	1	2	3	4	5
1	900	0*	0	2	8	35	8
2	50	0	0	1	9	4	3
3	0	0	0	0	6	4	3

*Colonies per gram of fecal material diluted one hundredfold.

A detectable Candida albicans population appeared in the two Aureomycin fed groups 48 hours after inoculation. None of these organisms were isolated in less than 72 hours from the untreated control group. A considerably higher population was observed in the group given the high level of Aureomycin than in the group given the low level. It was noted that although a detectable population was observed to build up in numbers more slowly in the untreated birds, the resulting population appeared to be nearly equal in magnitude to that of the group fed the low level of Aureomycin.

Two cockerels from each group were retained for a month after inoculation and observed for any outward symptoms of moniliasis. At the end of the period, they were sacrificed and their upper digestive tract examined for lesions which are thought to be typical of a Candida albicans infection. Nothing that resembled a lesion, either macroscopic or microscopic was found in any of the birds.

One of the more commonly reported symptoms of Candida albicans infection in the chicken is a general unthriftiness in as far as growth

and body maintance is concerned. An experiment was set up to determine if the C-1 strain would exert such an effect on chicks given a ration containing a low level of Aureomycin. At the same time, a parallel experiment was conducted to determine if the antifungal antibiotic nystatin fed in a concentration able to decrease a Candida albicans population to undetectable levels, could prevent any decrease in the rate of gain of the inoculated chicks. Studies with this antifungal agent had indicated that it can be used with some success in relieving the condition referred to as moniliasis (37).

The first step in this experiment consisted of finding the minimum amount of nystatin which could reduce a Candida albicans population to an undetectable level. The nine remaining cockerels of the control group of the preceeding experiment were used as the test birds. This group served as it's own control as the nine birds were shown to possess a well established Candida albicans population in their intestinal tracts.

Starting with the concentration of nystatin used by Yacowitz (45) to prevent the spread of experimental moniliasis, the amount of antibiotic was increased every three days until Candida albicans organisms could not be detected by the plating of fecal material in Sabouraud's agar.

The results of this experiment are contained in Table IV, page 31.

Using the concentration of nystatin which appeared to be effective in reducing an intestinal Candida albicans population to an undetectable level, the second step of this experiment was carried

TABLE IV. CANDIDA ALBICANS ISOLATED FROM NYSTATIN TREATED CHICKENS

Antibiotic content (units/gram)	Days			
	0	1	2	3
200	11*	10	13	9
250	-	6	4	5
300	-	1	0	0

*The number of colonies per gram of fecal material diluted one hundredfold. This number is an average of two plates.

out. Forty five one week old chicks were divided into four approximately equal groups and housed in separate cages. A ration considered adequate in all nutritional requirements, and supplemented with a high lvel of Aureomycin was fed to all four groups. The birds in group I were orally inoculated with viable Candida albicans in the same manner as described in the preceeding experiments. Group II was not inoculated as it was the control group for the first group. The third group of chicks were given a ration supplement of 300 units of nystatin per gram of feed in addition to being inoculated with viable cells. Group IV was also given the nystatin supplement, and served as a control group for the third group.*

Composite samples of fecal material from the chicks in each group were plated with Sabouraud's Maltose agar to insure that none of the groups were infected with Candida albicans prior to the start of this experiment. No such organisms were detected in any of the four groups.

*The nystatin was supplied by E.R. Squibb and Sons, Inc., New Brunswick, New Jersey.

The inoculated birds were housed in the bottom half of the battery to avoid any spread of this organism to the uninoculated birds, through scattering of contaminated droppings, feed or water. The control group were also checked weekly, to determine whether or not they had been infected since the start of the experiment. No detectable infection was observed in the two control groups during the duration of the experiment. The birds were weighed at the end of a 33 day period. The results of this experiment are enclosed in Table V.

TABLE V. THE EFFECT OF CANDIDA ALBICANS STRAIN C-1, IN THE PRESENCE OF AUREOMYCIN AND NYSTATIN ON THE GROWTH OF CHICKS

Group	Number of Chickens	Supplement	Inoculated	Total Weight	Average Weight Gain
1	11	Aureomycin (50 gms/ton)	+	4890*	441
2	11	Aureomycin (50 gms/ton)	-	5930	539
3	12	Aureomycin (50 gms/ton) and Nystatin (300 units/gm)	+	6410	534
4	11	Aureomycin (50 gms/ton) and Nystatin (300 units/gm)	-	6100	554

*Grams

The presence of the C-1 strain of Candida albicans in the

intestinal tracts of young chickens appeared to have a slightly depressive effect on their rate of gain. The birds, which were inoculated gained an average of 98 grams less than the control birds. Nystatin in concentration of 300 units per gram of ration appeared to be able to partially prevent this decreased rate of gain in this particular instance. Two chickens were sacrificed from each of the inoculated groups, and the esophagus, crop and proventriculus from each bird was examined for both macroscopic and microscopic lesions. None were found in any of the birds.

SUMMARY AND CONCLUSIONS

A microorganism which bore the macroscopic characteristics of the yeast like fungus Candida albicans was isolated from the intestinal tract of a normal appearing laying hen. The identity of this isolate was verified by inducing it to form an asexual spore referred to as a terminal chlamydospore. The formation of this relatively large, thick walled body in a medium devoid of reducing sugars is a characteristic peculiar to this yeastlike fungus only. In this study, the only sporulation medium which would stimulate this particular strain of Candida albicans to produce chlamydospores was a corn meal agar made with Quaker's Oats brand yellow corn meal. Another commercially prepared yellow corn meal, Argo, was unable to cause the formation of this asexual spore.

Sugar fermentation tests with two per cent carbohydrate solutions revealed that this strain designated C-1 and nine other isolates of Candida albicans were very similiar to those studied by Jungherr (25).

Of 14 commonly used antibiotics tested against the C-1 strain and nine other isolates of Candida albicans, only nystatin was able to inhibit the in vitro growth of these organisms. This antibiotic at a level of 50 grams per ton of ration seemed to be able to prevent natural infection of the intestinal tract of chickens as evidenced by plating of fecal samples from two groups of hens fed this antifungal agent.

Candida albicans was isolated in relatively large numbers from hens fed a low level of penicillin. However, hens fed this antibiotic

in combination with Terramycin showed comparably smaller populations. As this organism is highly resistant to this tetracycline and conditions of stress which are reported to aid infection were not peculiar to any one group, the cause of this difference in populations was looked for elsewhere. An attempt was made to explain this lesser Candida albicans population in the Terramycin fed birds in terms of antagonistic micro-organisms as reported by Sisburth et al (34) to be present in chicks and poults fed a high level of this wide spectrum antibiotic. Subsequent investigation revealed that the intestinal microflora antagonistic for Candida albicans were no more numerous in these birds than in those fed penicillin or no antibiotics at all.

Candida albicans or any other similar yeastlike fungus was not observed to voluntarily establish itself in the intestinal tracts of young chicks fed Aureomycin in both high and low levels and kept under conditions of seemly no stress. The C-1 strain when orally injected into these birds established itself more quickly in those chicks given the Aureomycin as compared to a control group which received no antibiotic supplement. Although the group of birds fed the higher level of Aureomycin possessed a larger average population, the group fed the lesser amount revealed a population of these organisms no longer than that of the control birds.

The only sign of infection by the C-1 strain other than the appearance of the organism in the feces and the mucous membranes lining the upper digestive tract was a slight decrease in the rate of weight gain of chicks which were orally inoculated with the organism. This effect was reversed by the addition of 300 units of nystatin to

each gram of ration.

It would appear from these studies that the only effect of low levels of Aureomycin on Candida albicans in the intestinal tracts of chickens is to facilitate its establishment in these organs. Apparently, there is no alteration of pathogenic properties in as far as injury to the mucous membranes of the upper digestive tract is concerned. What is not explained is the mode of action of the organism in causing the decreased rate of gain by chicks inoculated with the yeastlike fungus.

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