

1943

# The growth and cultural characteristics of pathogenic and nonpathogenic monolia on the chorio-allantoic membrane of the chick embryo

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The Growth and Cultural Characteristics of  
Pathogenic and Non-pathogenic Monilia on the  
Chorio-allantoic Membrane of the Chick Embryo

by

Eleanor Roberts Kinney

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BOSTON UNIVERSITY

GRADUATE SCHOOL

THESIS

The Growth and Cultural Characteristics of  
Pathogenic and Non-pathogenic Monilia on the  
Chorio-allantoic Membrane of the Chick Embryo

by

Eleanor Roberts Kinney

(A.B., Mount Holyoke College, 1936)

Submitted in partial fulfillment of  
the requirements for the degree of

Master of ~~Medical Sciences~~ Arts

1943



Approved

by

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~~Professor of~~





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- b. Strain 2 Obtained from culture of peritoneal cavity.



THE HISTORY OF THE

REIGN OF

CHARLES THE FIRST

IN THE

SEVENTEENTH CENTURY

BY

JOHN RICHARDSON

OF THE

UNIVERSITY OF

OXFORD

IN TWO VOLUMES

LONDON

PRINTED BY

JOHN RICHARDSON

AT THE

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IN THE

SEVENTEENTH CENTURY

BY

JOHN RICHARDSON

OF THE

UNIVERSITY OF

OXFORD

LONDON

c. Strain 3 Obtained from culture from sputum  
from patient with lung abscess.

2. Monilia krusei

Original culture from American Type Culture  
Collection.

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Original culture from American Type Culture  
Collection.

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- a. Organism.
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- c. After membrane passage.

3. M. bonordeni

- a. Organism.
- b. Histological pathology.
- c. After membrane passage.

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7. The seventh part is a report on the state of the Coast Guard.

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20. The twentieth part is a report on the state of the Department of the National Endowment for the Arts.

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25. The twenty-fifth part is a report on the state of the Department of the National Endowment for the Literature.

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The present thesis is based on a study of the growth characteristics of pathogenic and non-pathogenic *Monilia* on the chorio-allantoic membrane of the chick. The gross and histological appearance of the growth obtained and the lesions produced by the *Monilia* are described. The types of reaction produced on the chorio-allantoic membrane by three different species of *Monilia* are compared.

#### REVIEW OF LITERATURE

As far as is known the embryologists were the first to use fertile incubating hen's eggs. They recognized this medium as a convenient, easily adaptable and constantly available source in which to study developmental anatomy. Goodpasture<sup>(13)</sup> states that Beguelin<sup>(2)</sup> was the first to make a window in the egg shell so that the growing embryo could be observed during its development. Beguelin's method was to remove the shell and its membrane from the blunt end of the hen's egg during the early days of incubation. When the egg was not being studied, he would place over the opening a piece of shell which had been cut from the blunt end of another egg. Using this technic it was possible for him to remove the cover and so make observations whenever necessary. This work was re-

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ported before the Berlin Academy in 1749.

During the nineteenth century several other workers used similar methods and Scymkiewicz,<sup>(24)</sup> in 1815, had the ingenuity to cover the window in the shell by a coverglass, which was sealed with "wax" so that he was able to watch the developing embryo without disturbing it.

<sup>(8)</sup>  
Gerlach, in 1886, invented an instrument that he called the embryo-scope. It could be fixed in the shell opening and contained a removable piece of glass which allowed him to operate upon the growing embryo or to observe it through a microscope. The instrument was clumsy and never came into general use.

In 1898, Florence Peebles<sup>(21)</sup> also made windows in the shell thus permitting her to injure various portions of the primitive streak and to observe the subsequent development of the embryo. The method used by Peebles was a modification of Gerlach's technic.

It is interesting to note that the investigators mentioned above were all interested in problems of embryology and that any infection, which might by chance result from their manipulation of the embryo, would terminate or hinder their experiments.



Although egg contents have been employed for many years in the composition of media for bacteria, they were seldom used in making media for fungi. However, isolated early attempts were made to use the egg as a medium for the cultivation of fungi, for Wolff and Israel<sup>(26)</sup>, in 1891, injected purulent material removed from a retromaxillary nodule into raw and partially boiled hen's and pigeon's eggs and secured pure cultures of Actinomyces.

<sup>(15)</sup>  
Levaditi, in 1906, is generally credited with the first use of the developing chick embryo for the study of infection. In 1905, Borrel, according to Levaditi, injected into fertile eggs a small quantity of chicken blood containing spirilla of fowls and found that an acute septicemia, caused by the spirilla, of the embryo took place. These results were apparently not published by Borrel but in continuing the experiments, Levaditi perforated the shell of the egg with a needle, injected infected blood into the albumen of the egg and sealed the puncture hole. The striking thing about this experiment was that the spirilla would not grow unless the egg was fertile and contained a developing embryo. In other words, the presence of living embryonic tissue appeared to be necessary

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in order that infection by the spirillum could take place.

Rettger, <sup>(22)</sup>in 1913, studied the bacteriology of the hen's egg with special reference to its freedom from bacterial infection and found that the egg contents remained a sterile medium unless the eggs were subjected to moisture and dirt.

Murphy <sup>(20)</sup>was able successfully to graft the cells of certain mammalian tumors on to the chorio-allantoic membrane and was even able to transplant tumors from membrane to membrane. However, he was unable to get a successful transplant when newly hatched chicks were used. This experiment indicated that so far as tumors are concerned a change from a susceptible to an insusceptible state of the embryo occurred during its last two days of life within the shell. Stevenson, <sup>(25)</sup>however, attempted to graft human tumors on to the chick membrane without success.

Rous and Murphy <sup>(23)</sup>in studying the virus of the Rous' sarcoma of chickens demonstrated the value of the chorio-allantoic membrane of fertile eggs as a medium for experimental pathological problems as these investigators were able to produce growth of the sarcoma on the membrane.

Juan and Straub <sup>(14)</sup>in 1920 were able successfully to infect chick



embryos with the virus of avian pest. The inoculation was into the yolk sac and they were able to obtain six positive passages, but subsequent passages were negative.

Clark,<sup>(5)</sup> in 1920, in looking for a method by which his students in embryology could study problems which involved operations on chick embryos described the present sterile technic for making windows in the egg shell through which the embryo and its membranes could be observed and manipulated. This method has served as the foundation for the technic used by all later investigators.

In 1923, mention was made by Askanazy<sup>(1)</sup> of the production of tuberculous chicks by the infection of fertile eggs but little was done toward the development of the method. In 1929, Gay and Thompson<sup>(7)</sup> inoculated vaccinia virus into the yolk sac and recovered the virus in the second generation but the third transfer failed. In 1931, Woodruff and Goodpasture<sup>(27)</sup> published a paper on the susceptibility of the chorio-allantoic membrane of chick embryos to infection with fowl-pox virus modifying Clark's technic so that it could be used with consistent results. Goodpasture and his co-workers,<sup>(9, 10, 11, 12)</sup> particularly Buddingh, further



1. The first part of the report deals with the general situation of the country and the position of the various groups of the population. It is a very interesting and informative study of the social and economic conditions of the country.

2. The second part of the report deals with the results of the various surveys and studies conducted by the different departments of the government. It is a very detailed and comprehensive study of the various aspects of the country's development.

3. The third part of the report deals with the various projects and plans for the future development of the country. It is a very ambitious and far-reaching study of the various aspects of the country's development.

4. The fourth part of the report deals with the various conclusions and recommendations of the different departments of the government. It is a very detailed and comprehensive study of the various aspects of the country's development.

developed the technic until it came to be recognized as a reliable experimental procedure. This method was used by Goodpasture and his group and by other workers in the study of various viruses, bacteria, rickettsiae, and spirochetes.

In 1938, Goodpasture<sup>(13)</sup> mentioned the use of the developing chick membrane as a medium for fungal culture but published no formal communication on this work. Moore,<sup>(17, 18)</sup> in 1939, and in 1941, for the first time described the use of the chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. In 1941, Moore<sup>(19)</sup> published an additional paper on the use of the developing chick membrane for the cultivation of fungi with particular reference to Histoplasma capsulatum.



## TECHNIC FOR INOCULATION OF CHICK EMBRYOS

The technic used in the experiments described below was Goodpasture's modification of Clark's method. Fertile eggs were incubated at 37°C. in an ordinary bacteriological incubator and up to the time of inoculation were turned daily. Care was taken to keep air inside the incubator moist by placing two open beakers of water on the shelf of the incubator. On the day before the eggs were to be used, each egg was candled and the air space was outlined. When the eggs were transluminated during the candling, it was possible to identify the larger blood vessels on the membrane and a cross mark was placed over this area to designate the future site of the window. Eggs with non-viable embryos were discarded.

The shell covering the air sac was cleaned with 80 per cent alcohol. A slit, 4-6mm. in length, was cut in the shell, in order to expose the shell membrane, by means of a hand-electric drill with a circular revolving carburundum disc. The slit was coated with a sterile preparation consisting of three parts paraffin and one part petrolatum. The purpose of the "wax" was to prevent debris and powdered egg shell dust from falling into the air space. The shell membrane was then cut with a sharp

## THE HISTORY OF THE

REIGN OF KING CHARLES THE FIRST

IN WHICH ARE CONTAINED THE  
MOST IMPORTANT AND INTERESTING  
CIRCUMSTANCES OF HIS REIGN  
FROM HIS MARRIAGE TO HIS DEATH  
IN THE YEAR 1649  
BY  
JOHN BURNET  
BISHOP OF SALISBURY  
AND  
OF THE CHURCH OF ENGLAND  
IN THE PRESENT REIGN  
LONDON  
Printed by J. Sturges, at the Angel in St. Dunstons Church-yard, 1724

THE SECOND EDITION, CORRECTED.

IN TWO VOLUMES.  
THE FIRST VOLUME CONTAINS  
THE HISTORY OF HIS REIGN  
FROM HIS MARRIAGE TO HIS DEATH  
IN THE YEAR 1649  
THE SECOND VOLUME CONTAINS  
THE HISTORY OF HIS REIGN  
FROM HIS DEATH TO HIS BURIAL  
IN THE YEAR 1649  
LONDON  
Printed by J. Sturges, at the Angel in St. Dunstons Church-yard, 1724



sterile scalpel. Care was taken to insure free access of air into the air sac after the membrane had been cut. It was important that this opening was not occluded, otherwise the chorio-allantoic membrane would not drop away from the shell membrane when the window was cut in the shell.

A window, approximately 1 cm square, was then cut through the shell to the level of the shell membrane at the site previously determined. In doing this the egg was held in the hand and the window was cut with an electric drill. The shell had been washed previously with 80 per cent alcohol and the surface was painted with the sterile "wax". A clean linen towel was rolled in such a way as to form a support for the egg. An alternative method for holding the egg would be by means of a mold of plasticine. The shell membrane under the cut edge of the window was then cut using a sterile scalpel. In cutting the shell membrane great care must be taken not to injure the underlying chorio-allantoic membrane. Once the shell membrane was perforated in almost all cases gravity caused the membrane to fall. The shell window and shell membrane were removed by sterile pointed forceps and discarded. The window was rimmed with sterile petrolatum and a small coverglass was then flamed, allowed to





cool for a moment and placed on the petrolatum rim, partially melting the petrolatum and thus sealing the opening. Two alternatives were open:

(1) to inoculate the membrane before sealing with the coverslip, or (2) to seal the egg and place it in the incubator for 24 hours before inoculation.

The latter method had the advantage of allowing any embryos whose membranes had been badly traumatized to die before being inoculated, thus eliminating traumatic deaths from the experimental data.

The material to be cultured was placed directly on the membrane with a platinum loop. Once inoculated the eggs were placed in the incubator and observed daily through the coverglass. The embryos were killed after various predetermined intervals following inoculation. When the egg was to be sacrificed, the window was removed and a culture was made on Sabourraud's medium. The edge of the shell was cut down with scissors so that the upper third of the egg was exposed. Zenker's fluid and in some cases alcohol-formalin, was poured over the membrane and allowed to remain for four or five minutes. This method of treatment had the double advantage of giving instant fixation of tissues and of stiffening the membrane in situ so that it could be removed easily. The membrane bear-



ing the growth was then cut away with small iridectomy scissors and floated in sterile-isotonic saline solution. It was then floated on to a small piece of blotting paper in order to prevent the membrane's becoming wrinkled in the fixative. The membrane was fixed in Zenker's fluid or alcohol-formalin, sectioned in paraffin and stained with either phloxine-methylene blue or hematoxylin and eosin, and in some cases with Gram-Wiegert stain.



## SPECIES AND STRAINS OF MONILIA

1. A culture of Monilia albicans, which will be designated as Strain 1 in this paper, was obtained on April 1, 1940, from an ulcer of the hand of a male (B.C.H. Out-patient number 612,301). This organism was considered to be the causative agent in the patient's lesion. It was cultured on Sabourraud's medium and gave the usual reactions in sugars for M. albicans (table 10).
2. A strain of Monilia albicans which is designated Strain 2 was obtained at autopsy, (B.C.H. A-42-179), upon culture of the peritoneal cavity of an 81-year old male who died from peritonitis following a perforated chronic duodenal ulcer. This organism was accompanied by a Type 10 pneumococcus and an alpha type of streptococcus. The Monilia was transplanted to Sabourraud's medium and gave the usual reactions in sugars for M. albicans (table 10).
3. A strain of Monilia albicans, which is designated Strain 3, was obtained from a culture of sputum from a male suffering from a lung abscess, (B.C.H. Bacteria specimen B-42-5139). Alpha and alpha prime streptococci, diphtheroids, N. catarrhalis and N. pharyngis sicca were also obtained





from the sputum. The sugar reactions were characteristic of M. albicans (table 10).

4. A strain of Monilia krusei was obtained from the American Type Culture Collection. This species is usually considered as a non-pathogenic Monilia (table 10).

5. A strain of Monilia bonordeni was obtained from the American Type Culture Collection. This species is usually considered as a non-pathogenic Monilia (table 10).

#### EXPERIMENTAL RESULTS

##### 1. Monilia albicans

ORGANISM. Strain 1 of Monilia albicans was used to inoculate 31 eggs which had been previously incubated from 10 to 12 days. The eggs were observed daily and were sacrificed at times varying from 1 to 9 days following inoculation. The growth was rapid. By the end of 24 hours there were usually large, well-defined opaque masses of the organism. These were soft, piled-up, gray-white colonies from 0.5 to 4 cm in diameter, their size apparently varying directly with the concentration of the inoculum. These masses of organisms were firmly attached to the membrane and could



THE UNIVERSITY OF CHICAGO

1911

TO THE HONORABLE SENATE OF THE UNIVERSITY OF CHICAGO

IN RESPONSE TO A RESOLUTION PASSED AT THE MEETING OF THE SENATE

OF MAY 1, 1911

BY THE PRESIDENT OF THE UNIVERSITY OF CHICAGO

AND BY THE FACULTY OF THE UNIVERSITY OF CHICAGO

AND BY THE BOARD OF TRUSTEES

OF THE UNIVERSITY OF CHICAGO

CHICAGO, ILLINOIS

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not be dislodged without its rupture.

In each case it was possible to recover the organism on Sabouraud's medium or to produce growth by direct transplant to the membrane of a second egg.

The macroscopic growth characteristics of Strains 2 (10 eggs inoculated) and 3 (10 eggs inoculated) were identical with those of Strain 1.

In each case growth was recovered on Sabouraud's medium.

HISTOLOGICAL PATHOLOGY. Sections of the chorio-allantoic membrane were taken from the areas in which there was macroscopic growth of M. albicans.

The histological picture was the same for all strains of M. albicans studied as well as for those strains which had been transplanted from membrane to membrane. The colonies themselves were made up of masses of hyphae and spores. At the junction of the colonies and the membrane there was proliferation of the ectoderm. Here the proliferating ectoderm was invaded by a few monocytes, polymorphonuclear leukocytes, and spore forms of the fungi. In some of the sections in which growth had been allowed to progress there were nests of ectodermal-like cells which showed hyperkeratinization and resembled "epithelial pearls". These were particularly

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CHICAGO, ILLINOIS  
JANUARY 10, 1964  
DR. J. H. VAN VLIET  
1000 UNIVERSITY AVENUE  
CHICAGO, ILLINOIS 60607  
Dear Dr. Van Vliet:  
I have just received your letter of January 7, 1964, regarding the  
loan of a copy of the book "The History of the United States  
from 1789 to 1899" by John P. Kennedy. I am sorry that I  
cannot return the book to you at this time, but it is  
currently out of the library. I will return it to you as soon  
as it is available. I am sorry for the inconvenience.  
Very truly yours,  
J. H. VAN VLIET  
LIBRARIAN

prominent in sections inoculated with Strain 3. The ectodermal layer beneath the more central portion of the colony was either necrotic or could not be identified. A fine eosinophilic granular exudate covered the peripheral surface of the ectoderm and in this exudate could be seen red blood cells, monocytes, polymorphonuclear leukocytes, and cellular debris. In addition there were large masses of fungi present as spore forms and hyphae. The fungi grew in huge masses which extended down into the mesoderm and it was in the mesoderm that the most striking reaction took place.

The mesoderm was greatly thickened in width and this enlargement appeared to be due to edema, to congestion of the blood vessels, and to the presence of large masses of monocytes and granulocytes. Many of the granulocytes were eosinophilic. The fungi grew downward in large projections. At the edge of some of these projections was a margin of ectodermal-like cells giving a rather clear-cut border. Spore forms could be seen in this margin. However, in the majority of the sections this ectodermal-like border was absent and the lesions were much more diffuse and less well defined. There was necrosis in the areas of greatest infiltration. Scattered at the periphery of the masses was seen an occasional giant cell of





the Langhan's type. In some of these giant cells, spores were seen.

The entoderm was relatively uninvolved except in a few areas which were adjacent to marked mesodermal involvement and in these areas there was focal proliferation of the ectoderm together with slight leukocytic infiltration.

## 2. Monilia krusei

ORGANISM. Monilia krusei was used to inoculate a series of 31 eggs.

Growth occurred in each case. The organisms grew in discrete colonies which were piled up and soft, and opaque and gray-white in color. They were loosely adherent to the membrane and could be easily pulled away leaving an intact membrane. Growth was rapid during the first 24 hours and the colonies grew somewhat more slowly during the subsequent 24 hours. By the end of 48 hours the colonies averaged 0.5 cm in diameter. The colonies then began to regress and by the end of the next 5 days they had regressed to almost half their former size and were dry and scaly. In some cases, if the process was allowed to go on, the growth would entirely disappear. M. krusei after passage through one membrane was transplanted to other membranes in 11 cases. The colonies were larger but otherwise

The following table shows the results of the survey conducted in 1998. The data is presented in a table format with columns for the year, the number of respondents, and the percentage of respondents who answered 'yes' to the question 'Do you support the proposed changes to the law?'.

### Table 1: Survey Results (1998)

The survey was conducted in 1998 and the results are presented in the table below. The table shows the number of respondents and the percentage of respondents who answered 'yes' to the question 'Do you support the proposed changes to the law?'.

Year	Number of Respondents	Percentage of 'Yes' Answers
1998	100	75%

The results of the survey indicate that 75% of the respondents supported the proposed changes to the law. This is a significant majority and suggests that the proposed changes are well-received by the public.

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were similar to those described above for M. krusei.

HISTOLOGICAL PATHOLOGY. Microscopic sections were taken from areas in which colonies of M. krusei were grossly identified. The periphery of the colonies appeared to be loosely attached to the ectoderm and the colonies were made up for the greater part of masses of spores with only a few hyphae. Much of the ectoderm beneath the colonies showed no reaction but the ectoderm at the center of the colonies usually showed focal areas of proliferation containing occasional spore forms together with a few monocytes and polymorphonuclear leukocytes. The mesoderm for the greater part was uninvolved but focal areas contained a few polymorphonuclear leukocytes and monocytes. The entoderm was not involved.

Sections of membranes inoculated with organisms which had been re-activated and recovered from growth on a previous chick embryo membrane were studied. The reaction was much more marked. Microscopically the colonies did not appear to be as loosely attached as previously described and the ectoderm throughout was invaded by spore forms and infiltrated by monocytes and polymorphonuclear leukocytes. In some areas the ectoderm had sloughed. The mesoderm was thickened and edematous and there was fi-

The American Medical Association is a non-profit corporation organized for the purpose of promoting the interests of the medical profession and the public. It was organized in 1847 and has since that time been the leading organization of the medical profession in the United States. The Association is composed of more than 50,000 members, who are organized into local, state, and national societies. The Association's principal activities are the publication of the Journal of the American Medical Association, the holding of annual meetings, and the promotion of medical education and research. The Association also maintains a large library and a museum of medical history.

The Journal of the American Medical Association is published weekly, except on Sundays and public holidays. It is the largest medical journal in the United States and is read by more than 100,000 physicians.

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The Association's principal publications, other than the Journal, are the American Medical News, the American Medical Journal, and the American Medical Review.

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broblastic proliferation. The blood vessels were distended by nucleated red blood cells. In addition there was diffuse infiltration by polymorphonuclear leukocytes, many eosinophilic cells and a few monocytes. There was no evidence of giant cell formation. In areas in which the mesodermal lesion was most marked there was proliferation of the entoderm.

Sections were taken from membranes upon which there was regression of the colonies. These showed essentially the same histological picture as described above except that the colonies were smaller and the lesions more superficial. In addition there were relatively more monocytes present and there were areas in the mesoderm in which young fibrous tissue had been deposited. Of particular interest were the relatively large numbers of clusters of ectodermal-like cells showing hyperkeratinization. These structures resembled "epithelial pearls".

### 3. Monilia bonordeni

ORGANISM. A group of membranes of 37 eggs were inoculated with Monilia bonordeni. The colonies grew steadily for 48 hours following inoculation. As in the case of Monilia krusei, the colonies then began to decrease in size so that by the end of 5 days they were definitely smaller and dried





out. However, it was always possible to recover the organism on Sabouraud's medium. At the height of their growth, the colonies were round, gray-white, opaque, discrete, and easily detachable from the membrane.

Thirteen eggs were inoculated with M. bonordeni which had been reactivated by passage through previous membranes. The gross cultural characteristics were similar to those described.

HISTOLOGICAL PATHOLOGY. Sections of membrane were taken so as to transect the colonies of M. bonordeni. Colonies were made up of large masses of fungi with many hyphae. The spores were especially prominent. The ectoderm for the most part had sloughed but in those areas in which it remained, it had proliferated and was infiltrated by spore forms, polymorphonuclear leukocytes and monocytes. In sections in which growth had occurred for 48 hours or longer there were groups of ectodermal cells arranged in round nests. These cells showed hyperkeratinization and resembled "epithelial pearls". The mesodermal layer was swollen and diffusely infiltrated by polymorphonuclear leukocytes and mononuclear cells. Many of the granulocytes took an eosin stain. There were spores and hyphae present in the lesion. The blood vessels were congested with red





blood cells. There was slight proliferation of the entoderm in the areas adjacent to the inflamed mesoderm.

Sections for study were taken from membranes which had been inoculated with organisms reactivated and recovered from previous chick embryo membranes. Here the reaction was similar to that previously described but seemed to be more severe. Beneath many of the colonies and extending into the mesoderm were areas of necrosis in which there were masses of polymorphonuclear leukocytes, monocytes, and red blood cells, spore forms and some hyphae.

Sections from membranes in which the growth showed macroscopic regression were studied. The colonies appeared to be but loosely attached to the membrane. There were areas in the mesoderm in which young fibrous tissue had been laid down. The majority of the granulocytes and particularly those taking an eosin stain had disappeared leaving the monocyte as the predominant cell. The ectoderm showed marked proliferation and the lesions seemed to be more superficial than those of the younger stages.

#### 4. PATHOGENICITY

In an effort to secure an approximate estimate of the pathogenicity

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of these three species of *Monilia*, it was decided to determine the mortality among the embryos at an arbitrary period of time. Forty-eight hours was chosen as the figure. It was found that Strain 1 of *M. albicans* caused a mortality of 37 per cent and that after passage through one membrane, the mortality rose to 45 per cent (tables 1-9). *M. albicans*, Strain 2, gave a mortality of 70 per cent and Strain 3 of *M. albicans* gave a mortality of 60 per cent. The mortality figure for *M. krusei* was 21 per cent while that for *M. bonordeni* was 26 per cent. When *M. krusei* and *M. bonordeni* were passed through one membrane, the mortality rose to 55 per cent and 31 per cent respectively.

#### DISCUSSION

ORGANISMS. Representative sections of colonies of *M. albicans*, *M. krusei*, *M. bonordeni* were stained by the Gram-Weigert technic. In the colonies of *M. albicans* on the surface of the chick membrane were thin-walled and slender branching hyphae with chlamydospores at the tips of the branches. In addition there were masses of oval, budding spores which were most numerous around the edges of the colonies. Again, in the lesions produced by this organism, both hyphae and budding forms were present and here the

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budding forms were most numerous at the periphery of the lesion. It is interesting to note that a giant cell reaction was stimulated and that some of the budding forms had been enveloped by the giant cells.

In the colonies of M. krusei the hyphae appeared as hairlike threads with branching at wider intervals. There were both hyphae and bud forms in the lesions. No chlamydospore-like bodies were present. The budding forms were much larger and more oval than those of M. albicans. The proportion of spore forms to hyphae was greater in M. krusei than M. albicans.

In the colonies of M. bonordeni the hyphae appeared as thin hairs. No branching or chlamydospores were seen. The budding forms were relatively large, elongated and quite numerous. Both forms were present in the lesion though the budding forms predominated. The findings were consistent enough to permit the identification of each organism by the appearance of the hyphae and spores.

PATHOLOGY. The lesions produced by the three organisms were essentially the same and differed in degree rather than kind. The lesions produced by M. albicans were more severe than those produced by the other two. The M. albicans produced more necrosis and the lesions were of greater depth.



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Giant cell formation was seen in lesions produced by M. albicans. No giant cells were seen in lesions produced by the first and second generations of M. krusei and the first generation of M. bonordeni. It is of interest to note that the lesions of M. bonordeni were more severe and that there was giant cell formation after the passage of the organism through one membrane.

PATHOGENICITY. The mortality of the embryos after forty-eight hours using fungus obtained from culture averaged 38-70 per cent for M. albicans; 21 per cent for M. krusei; and 26 per cent for M. bonordeni. Confirmation of the fact that the lesions produced by M. albicans were more severe is that it was not uncommon for colonies of M. krusei and M. bonordeni to regress in size or even disappear. The mortality figures deserve comment. It is interesting to note that Strain 1 of M. albicans gave mortality of only 38 per cent while both Strains 2 and 3 caused a mortality of 70 per cent and 60 per cent respectively. Also, after the passage of Strain 1 through an egg membrane, the mortality rose to 45 per cent. It is possible that the discrepancy between the low mortality rate of Strain 1 and of Strain 2 and 3 is due to the fact that Strain 1 had been carried on Sab-



ouraud's medium for a period of years while Strains 2 and 3 were freshly isolated. Further confirmation of this suggestion is the increase of the mortality rate from 38 to 45 per cent after the passage of the fungus through but one egg membrane. It is also interesting to note the increase in mortality rate and severity of the lesions after the passage of M. krusei and M. bonordeni through membranes. It would seem that the chorio-allantoic membrane of the developing chick would offer a readily available means for increasing the virulence of these organisms.

Moore<sup>(18)</sup> was impressed with the formation of the epithelial pearls in the mesoderm after the inoculation of the membrane with M. albicans. He states "this process was analogous in all respects to that in infection of human epithelium with the same organism". \* These structures were found in the present series but were present not only in the membranes inoculated with M. albicans but also in those membranes inoculated with M. krusei and M. bonordeni. Emmart and Smith<sup>(6)</sup> produced similar "pearls" by injection of tuberculin and by the implantation of tubercle bacilli on the chorio-allantoic membrane. Moore<sup>(19)</sup> describes ectodermal pearls in the

\* M. Moore, "The Chorio-allantoic Membrane of the Developing Chick as a Medium for the Cultivation and Histopathologic Study of Pathogenic Fungi". Am. J. Path., 1941, 17, p.112.

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mesoderm after the implantation of Histoplasma capsulatum. Canat and Opie<sup>(3, 4)</sup> demonstrated proliferation of ectodermal cells with the formation of papilla-like projections with abnormal keratinization of cells following the introduction of carbon particles and turpentine. It would seem that the formation of pearls and the keratinization and hyperplasia of ectoderm is not a specific reaction to M. albicans but rather is a non-specific reaction on the part of the membrane to any type of irritant.

#### SUMMARY

1. A comparative study of the cultural characteristics of three strains of Monilia albicans, one strain of Monilia bonordeni, and one strain of Monilia krusei on the chorio-allantoic membrane of chick embryos has been made.
2. The lesions in the membrane produced by the various species are described and discussed.
3. It is suggested that passage through the chorio-allantoic membrane causes the virulence of these organisms to be increased.
4. It is concluded that the pathological reactions to these organisms are similar but that the pathogen, Monilia albicans, causes lesions





which are much more severe in degree than those of the questionable non-pathogens, Monilia bonordeni and Monilia krusei.

1. The first part of the document is a letter from the President of the United States to the Congress.

2. The second part is a report from the Secretary of the Treasury.

3. The third part is a report from the Secretary of the Interior.

## TABULATION OF EXPERIMENTAL DATA

TABLE 1

Mortality of Chick Embryos after 48 HoursMonilia albicans

## Strain 1

- A. Obtained from culture.....38 per cent  
 B. After membrane passage.....45 per cent

## Strain 2

- A. Obtained from culture.....70 per cent

## Strain 3

- A. Obtained from culture.....60 per cent

Monilia krusei

- A. Obtained from culture.....21 per cent  
 B. After membrane passage.....55 per cent

Monilia bonordeni

- A. Obtained from culture.....26 per cent  
 B. After membrane passage.....31 per cent

SYMBOLS--In tables 2-10 the following symbols have been used:

<u>Growth</u>	<u>State of Embryo</u>	<u>Acid and Gas</u>
+ = Slight	D = Dead	A sl = Slightly acid
++ = Moderate	A = Alive	G numerals = percentage
+++ = Extensive		of gas
++++ = Luxuriant		
R = Regression		

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TABLE 2

Monilia albicans, Strain 1 (Obtained from culture)

Number of Egg	Growth			Final Growth		Growth of Embryo	Microscopic Recovered Sections
	48 hours	120 hours	Final	Time(Days)	State		
1	++	+++	++++	6	A	Yes	Yes
2			++	1	D	Yes	No
18	++		++	3	D	Yes	Yes
19	+++		+++	3	D	Yes	Yes
21	+		+	3	D	Yes	Yes
22	++	+++	+++	9	A	Yes	Yes
46	++	+++	+++	5	D	Yes	No
47	++	+++	+++	5	A	Yes	Yes
48	++	+++	+++	5	A	Yes	No
50	++	+++	+++	5	D	Yes	Yes
51			+	1	D	Yes	No
126			+++	1	D	Yes	No
127	+++		+++	2	D	Yes	No
128	++		+++	3	D	Yes	No
129			++	1	D	Yes	No
130			++	1	D	Yes	No





TABLE 3

Monilia albicans, Strain 1 (After membrane passage)

Number of Egg	Cultured from Egg	Growth		Final Growth	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
		48 Hours	120 Hours	Final				
20	1	++	+++	+++	7	D	Yes	Yes
23	1	++	+++	+++	7	D	Yes	Yes
33	1	+	++	++	5	D	Yes	Yes
34	1	+	+	+	9	D	Yes	Yes
35	1	+	++	++	5	D	Yes	No
36	1	+	++	+++	5	D	Yes	Yes
37	1	+	++	+++	5	D	Yes	Yes
38	1	++	++	+++	5	D	Yes	No
131	1	++	++	+++	2	D	Yes	No
132	1	++	++	+++	2	D	Yes	No
133	1			++	1	D	Yes	No
134	1			++	1	D	Yes	No
135	1	++		++	2	D	Yes	No
136	1			++	1	D	Yes	No
137	1	+++		+++	2	D	Yes	No

# Table

Table 1. Summary of the data for the first 1000 cases.

Case No.	Age	Sex	Occupation	Residence	Onset Date	Onset Time	Duration	Outcome
1	25	M	Student	Urban	1990-01-01	10:00	10	Recovered
2	30	F	Teacher	Rural	1990-01-05	12:00	15	Recovered
3	28	M	Farmer	Urban	1990-01-10	08:00	12	Recovered
4	35	F	Homemaker	Rural	1990-01-15	14:00	18	Recovered
5	22	M	Student	Urban	1990-01-20	09:00	8	Recovered
6	32	F	Teacher	Rural	1990-01-25	11:00	14	Recovered
7	27	M	Farmer	Urban	1990-02-01	07:00	11	Recovered
8	38	F	Homemaker	Rural	1990-02-05	13:00	19	Recovered
9	23	M	Student	Urban	1990-02-10	08:00	9	Recovered
10	33	F	Teacher	Rural	1990-02-15	12:00	16	Recovered
11	29	M	Farmer	Urban	1990-02-20	09:00	13	Recovered
12	36	F	Homemaker	Rural	1990-02-25	14:00	20	Recovered
13	24	M	Student	Urban	1990-03-01	07:00	10	Recovered
14	31	F	Teacher	Rural	1990-03-05	11:00	15	Recovered
15	26	M	Farmer	Urban	1990-03-10	08:00	11	Recovered
16	34	F	Homemaker	Rural	1990-03-15	13:00	18	Recovered
17	21	M	Student	Urban	1990-03-20	09:00	7	Recovered
18	37	F	Teacher	Rural	1990-03-25	12:00	17	Recovered
19	28	M	Farmer	Urban	1990-04-01	07:00	12	Recovered
20	39	F	Homemaker	Rural	1990-04-05	14:00	21	Recovered
21	25	M	Student	Urban	1990-04-10	08:00	9	Recovered
22	32	F	Teacher	Rural	1990-04-15	11:00	16	Recovered
23	27	M	Farmer	Urban	1990-04-20	09:00	13	Recovered
24	35	F	Homemaker	Rural	1990-04-25	13:00	19	Recovered
25	23	M	Student	Urban	1990-05-01	07:00	10	Recovered
26	31	F	Teacher	Rural	1990-05-05	12:00	15	Recovered
27	26	M	Farmer	Urban	1990-05-10	08:00	11	Recovered
28	34	F	Homemaker	Rural	1990-05-15	14:00	20	Recovered
29	21	M	Student	Urban	1990-05-20	09:00	7	Recovered
30	37	F	Teacher	Rural	1990-05-25	12:00	17	Recovered
31	28	M	Farmer	Urban	1990-06-01	07:00	12	Recovered
32	39	F	Homemaker	Rural	1990-06-05	14:00	21	Recovered
33	25	M	Student	Urban	1990-06-10	08:00	9	Recovered
34	32	F	Teacher	Rural	1990-06-15	11:00	16	Recovered
35	27	M	Farmer	Urban	1990-06-20	09:00	13	Recovered
36	35	F	Homemaker	Rural	1990-06-25	13:00	19	Recovered
37	23	M	Student	Urban	1990-07-01	07:00	10	Recovered
38	31	F	Teacher	Rural	1990-07-05	12:00	15	Recovered
39	26	M	Farmer	Urban	1990-07-10	08:00	11	Recovered
40	34	F	Homemaker	Rural	1990-07-15	14:00	20	Recovered
41	21	M	Student	Urban	1990-07-20	09:00	7	Recovered
42	37	F	Teacher	Rural	1990-07-25	12:00	17	Recovered
43	28	M	Farmer	Urban	1990-08-01	07:00	12	Recovered
44	39	F	Homemaker	Rural	1990-08-05	14:00	21	Recovered
45	25	M	Student	Urban	1990-08-10	08:00	9	Recovered
46	32	F	Teacher	Rural	1990-08-15	11:00	16	Recovered
47	27	M	Farmer	Urban	1990-08-20	09:00	13	Recovered
48	35	F	Homemaker	Rural	1990-08-25	13:00	19	Recovered
49	23	M	Student	Urban	1990-09-01	07:00	10	Recovered
50	31	F	Teacher	Rural	1990-09-05	12:00	15	Recovered
51	26	M	Farmer	Urban	1990-09-10	08:00	11	Recovered
52	34	F	Homemaker	Rural	1990-09-15	14:00	20	Recovered
53	21	M	Student	Urban	1990-09-20	09:00	7	Recovered
54	37	F	Teacher	Rural	1990-09-25	12:00	17	Recovered
55	28	M	Farmer	Urban	1990-10-01	07:00	12	Recovered
56	39	F	Homemaker	Rural	1990-10-05	14:00	21	Recovered
57	25	M	Student	Urban	1990-10-10	08:00	9	Recovered
58	32	F	Teacher	Rural	1990-10-15	11:00	16	Recovered
59	27	M	Farmer	Urban	1990-10-20	09:00	13	Recovered
60	35	F	Homemaker	Rural	1990-10-25	13:00	19	Recovered
61	23	M	Student	Urban	1990-11-01	07:00	10	Recovered
62	31	F	Teacher	Rural	1990-11-05	12:00	15	Recovered
63	26	M	Farmer	Urban	1990-11-10	08:00	11	Recovered
64	34	F	Homemaker	Rural	1990-11-15	14:00	20	Recovered
65	21	M	Student	Urban	1990-11-20	09:00	7	Recovered
66	37	F	Teacher	Rural	1990-11-25	12:00	17	Recovered
67	28	M	Farmer	Urban	1990-12-01	07:00	12	Recovered
68	39	F	Homemaker	Rural	1990-12-05	14:00	21	Recovered
69	25	M	Student	Urban	1990-12-10	08:00	9	Recovered
70	32	F	Teacher	Rural	1990-12-15	11:00	16	Recovered
71	27	M	Farmer	Urban	1990-12-20	09:00	13	Recovered
72	35	F	Homemaker	Rural	1990-12-25	13:00	19	Recovered
73	23	M	Student	Urban	1991-01-01	07:00	10	Recovered
74	31	F	Teacher	Rural	1991-01-05	12:00	15	Recovered
75	26	M	Farmer	Urban	1991-01-10	08:00	11	Recovered
76	34	F	Homemaker	Rural	1991-01-15	14:00	20	Recovered
77	21	M	Student	Urban	1991-01-20	09:00	7	Recovered
78	37	F	Teacher	Rural	1991-01-25	12:00	17	Recovered
79	28	M	Farmer	Urban	1991-02-01	07:00	12	Recovered
80	39	F	Homemaker	Rural	1991-02-05	14:00	21	Recovered
81	25	M	Student	Urban	1991-02-10	08:00	9	Recovered
82	32	F	Teacher	Rural	1991-02-15	11:00	16	Recovered
83	27	M	Farmer	Urban	1991-02-20	09:00	13	Recovered
84	35	F	Homemaker	Rural	1991-02-25	13:00	19	Recovered
85	23	M	Student	Urban	1991-03-01	07:00	10	Recovered
86	31	F	Teacher	Rural	1991-03-05	12:00	15	Recovered
87	26	M	Farmer	Urban	1991-03-10	08:00	11	Recovered
88	34	F	Homemaker	Rural	1991-03-15	14:00	20	Recovered
89	21	M	Student	Urban	1991-03-20	09:00	7	Recovered
90	37	F	Teacher	Rural	1991-03-25	12:00	17	Recovered
91	28	M	Farmer	Urban	1991-04-01	07:00	12	Recovered
92	39	F	Homemaker	Rural	1991-04-05	14:00	21	Recovered
93	25	M	Student	Urban	1991-04-10	08:00	9	Recovered
94	32	F	Teacher	Rural	1991-04-15	11:00	16	Recovered
95	27	M	Farmer	Urban	1991-04-20	09:00	13	Recovered
96	35	F	Homemaker	Rural	1991-04-25	13:00	19	Recovered
97	23	M	Student	Urban	1991-05-01	07:00	10	Recovered
98	31	F	Teacher	Rural	1991-05-05	12:00	15	Recovered
99	26	M	Farmer	Urban	1991-05-10	08:00	11	Recovered
100	34	F	Homemaker	Rural	1991-05-15	14:00	20	Recovered

TABLE 4

Monilia albicans, Strain 2 (Obtained from culture)

Number of Egg	Growth			Final Growth		Growth Recovered	Microscopic Sections
	48 hours	120 hours	Final	Time (Days)	State of Embryo		
58	++	+++	+++	7	D	Yes	Yes
59	++	+++	++++	7	D	Yes	No
60			+	1	D	Yes	No
61	++		++	2	D	Yes	Yes
62	+		+	2	D	Yes	Yes
149			+	1	D	Yes	No
150	++		+++	3	D	Yes	No
151	++		++	2	D	Yes	No
152			+	1	D	Yes	No
153			+	1	D	Yes	No





TABLE 5

Monilia albicans, Strain 3 (Obtained from culture)

Number of Egg	Growth			Final Growth			
	48 hours	120 hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
63	+	++	++	7	A	Yes	No
64	++	+++	++++	14	A	Yes	Yes
65	++	+++	++++	14	D	Yes	Yes
66	++	+++	++++	7	A	Yes	Yes
67			+	1	D	Yes	No
143	++		++	2	D	Yes	No
144	++		++	2	D	Yes	No
145			++	1	D	Yes	No
147	+		+	1	D	Yes	No
148	++		++	1	D	Yes	No

Date	Time	Temperature		Wind		Direction	Remarks
		Air	Water	Force	Direction		
10	08	75	75	10	SE	-	1
10	10	75	75	10	SE	SE	2
10	12	75	75	10	SE	SE	3
10	14	75	75	10	SE	SE	4
10	16	75	75	10	SE	SE	5
10	18	75	75	10	SE	SE	6
10	20	75	75	10	SE	SE	7
10	22	75	75	10	SE	SE	8
10	24	75	75	10	SE	SE	9
10	26	75	75	10	SE	SE	10
10	28	75	75	10	SE	SE	11
10	30	75	75	10	SE	SE	12

TABLE 6

Monilia krusei (Obtained from culture)

Number of Egg	Growth			Final Growth			
	48 hours	120 hours	Final	Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
68	++	R	+	12	A	Yes	Yes
70	++	R	+	10	A	Yes	Yes
71	+		+	12	D	Yes	No
72	+++	R	+	12	D	No	No
73	++	R	+	7	D	Yes	Yes
74	+++	R	+	12	A	Yes	Yes
96	++	R	+	10	A	Yes	Yes
97	++	R	+	10	A	Yes	Yes
98	+	R	+	2	A	Yes	No
99	++	R	+	2	D	Yes	Yes
154			+	1	D	Yes	No
155	++	R	+	4	D	Yes	No
156			+	1	D	Yes	No
187			++	1	A *	Yes	Yes
188	+		+	2	D	Yes	No
189	++	R	+	6	A	Yes	Yes
197	++		++	2	A	Yes	Yes
198	++	R	+	6	A	Yes	Yes
199	++		++	3	A	Yes	Yes
200	++	R	Dis- appeared	6	A	No	Yes

\* Killed for 24 hour section. Not considered in mortality rate.



TABLE 7

Monilia krusei (After membrane passage)

Number of Egg	Culture from Egg	Growth			Final Growth		Growth Recovered	Microscopic Sections
		48 Hours	120 Hours	Final	Time (Days)	State of Embryo		
108	73	++		+++	7	D	Yes	Yes
110	74	++		+++	10	A	Yes	Yes
111	68	++		++	2	D	Yes	Yes
112	68	++		++	2	D	Yes	Yes
113	70	++		++	2	D	Yes	Yes
114	70	++		+++	10	A	Yes	Yes
123	71	++		+++	10	A	Yes	Yes
125	70	++		++	2	D	Yes	Yes
168	68			++	1	D	Yes	No
169	73	++		++	4	D	Yes	No
170	73			++	1	D	Yes	No



# Table 1. Summary of data for the study.

Year	Month	Day	Time	Location	Temperature (°C)	Humidity (%)	Wind Speed (m/s)
2018	Jan	1	10:00	10	15	60	1.2
2018	Jan	2	10:00	10	16	62	1.3
2018	Jan	3	10:00	10	17	65	1.4
2018	Jan	4	10:00	10	18	68	1.5
2018	Jan	5	10:00	10	19	70	1.6
2018	Jan	6	10:00	10	20	72	1.7
2018	Jan	7	10:00	10	21	75	1.8
2018	Jan	8	10:00	10	22	78	1.9
2018	Jan	9	10:00	10	23	80	2.0
2018	Jan	10	10:00	10	24	82	2.1
2018	Jan	11	10:00	10	25	85	2.2
2018	Jan	12	10:00	10	26	88	2.3
2018	Jan	13	10:00	10	27	90	2.4
2018	Jan	14	10:00	10	28	92	2.5
2018	Jan	15	10:00	10	29	95	2.6
2018	Jan	16	10:00	10	30	98	2.7
2018	Jan	17	10:00	10	31	100	2.8
2018	Jan	18	10:00	10	32	100	2.9
2018	Jan	19	10:00	10	33	100	3.0
2018	Jan	20	10:00	10	34	100	3.1
2018	Jan	21	10:00	10	35	100	3.2
2018	Jan	22	10:00	10	36	100	3.3
2018	Jan	23	10:00	10	37	100	3.4
2018	Jan	24	10:00	10	38	100	3.5
2018	Jan	25	10:00	10	39	100	3.6
2018	Jan	26	10:00	10	40	100	3.7
2018	Jan	27	10:00	10	41	100	3.8
2018	Jan	28	10:00	10	42	100	3.9
2018	Jan	29	10:00	10	43	100	4.0
2018	Jan	30	10:00	10	44	100	4.1
2018	Jan	31	10:00	10	45	100	4.2

TABLE 8

Monilia bonordeni (Obtained from culture)

Number of Egg	Growth		Final Growth		State of Embryo	Growth Recovered	Microscopic Sections
	48 hours	120 hours	Final	Time (Days)			
75	+		+	2	D	Yes	Yes
76	+		+	2	D	Yes	Yes
77	+		+	2	D	Yes	Yes
78	++	R	++	10	A	Yes	Yes
79	++	R	++	12	A	Yes	Yes
80	++	R	++	7	D	Yes	Yes
81	++	R	+	12	D	Yes	Yes
82	++	R	++	10	A	Yes	Yes
100	++		++	2	D	Yes	Yes
101	+++	R	++	10	A	Yes	Yes
102	++	R	+	7	D	Yes	Yes
103	++	R	+	4	D	Yes	Yes
177	++	R	+	4	D	Yes	No
178	++	R	+	4	D	Yes	No
179	++		++	4	D	Yes	No
180	++		++	2	D	Yes	No
181			+	1	D	Yes	No
190			++	1	A	Yes *	Yes
191	++		++	2	A	Yes	Yes
192	++	R	+	6	A	Yes	Yes
193	++	R	+	6	A	Yes	Yes
194	++	R	+	6	A	Yes	Yes
195	++		++	3	A	Yes	Yes
196	++	R	+	6	A	Yes	Yes

\*Killed for 24 hour section. Not considered in mortality rate.

# Table 1. Summary of data for the 1990-1991 season.

Year	Month	Day	Time	Location	Species	Count	Notes
1990	Jan	1	10:00	1000	1	1	
1990	Jan	2	10:00	1000	2	2	
1990	Jan	3	10:00	1000	3	3	
1990	Jan	4	10:00	1000	4	4	
1990	Jan	5	10:00	1000	5	5	
1990	Jan	6	10:00	1000	6	6	
1990	Jan	7	10:00	1000	7	7	
1990	Jan	8	10:00	1000	8	8	
1990	Jan	9	10:00	1000	9	9	
1990	Jan	10	10:00	1000	10	10	
1990	Jan	11	10:00	1000	11	11	
1990	Jan	12	10:00	1000	12	12	
1990	Jan	13	10:00	1000	13	13	
1990	Jan	14	10:00	1000	14	14	
1990	Jan	15	10:00	1000	15	15	
1990	Jan	16	10:00	1000	16	16	
1990	Jan	17	10:00	1000	17	17	
1990	Jan	18	10:00	1000	18	18	
1990	Jan	19	10:00	1000	19	19	
1990	Jan	20	10:00	1000	20	20	
1990	Jan	21	10:00	1000	21	21	
1990	Jan	22	10:00	1000	22	22	
1990	Jan	23	10:00	1000	23	23	
1990	Jan	24	10:00	1000	24	24	
1990	Jan	25	10:00	1000	25	25	
1990	Jan	26	10:00	1000	26	26	
1990	Jan	27	10:00	1000	27	27	
1990	Jan	28	10:00	1000	28	28	
1990	Jan	29	10:00	1000	29	29	
1990	Jan	30	10:00	1000	30	30	
1990	Jan	31	10:00	1000	31	31	
1990	Feb	1	10:00	1000	32	32	
1990	Feb	2	10:00	1000	33	33	
1990	Feb	3	10:00	1000	34	34	
1990	Feb	4	10:00	1000	35	35	
1990	Feb	5	10:00	1000	36	36	
1990	Feb	6	10:00	1000	37	37	
1990	Feb	7	10:00	1000	38	38	
1990	Feb	8	10:00	1000	39	39	
1990	Feb	9	10:00	1000	40	40	
1990	Feb	10	10:00	1000	41	41	
1990	Feb	11	10:00	1000	42	42	
1990	Feb	12	10:00	1000	43	43	
1990	Feb	13	10:00	1000	44	44	
1990	Feb	14	10:00	1000	45	45	
1990	Feb	15	10:00	1000	46	46	
1990	Feb	16	10:00	1000	47	47	
1990	Feb	17	10:00	1000	48	48	
1990	Feb	18	10:00	1000	49	49	
1990	Feb	19	10:00	1000	50	50	
1990	Feb	20	10:00	1000	51	51	
1990	Feb	21	10:00	1000	52	52	
1990	Feb	22	10:00	1000	53	53	
1990	Feb	23	10:00	1000	54	54	
1990	Feb	24	10:00	1000	55	55	
1990	Feb	25	10:00	1000	56	56	
1990	Feb	26	10:00	1000	57	57	
1990	Feb	27	10:00	1000	58	58	
1990	Feb	28	10:00	1000	59	59	
1990	Feb	29	10:00	1000	60	60	
1990	Feb	30	10:00	1000	61	61	
1990	Feb	31	10:00	1000	62	62	
1990	Mar	1	10:00	1000	63	63	
1990	Mar	2	10:00	1000	64	64	
1990	Mar	3	10:00	1000	65	65	
1990	Mar	4	10:00	1000	66	66	
1990	Mar	5	10:00	1000	67	67	
1990	Mar	6	10:00	1000	68	68	
1990	Mar	7	10:00	1000	69	69	
1990	Mar	8	10:00	1000	70	70	
1990	Mar	9	10:00	1000	71	71	
1990	Mar	10	10:00	1000	72	72	
1990	Mar	11	10:00	1000	73	73	
1990	Mar	12	10:00	1000	74	74	
1990	Mar	13	10:00	1000	75	75	
1990	Mar	14	10:00	1000	76	76	
1990	Mar	15	10:00	1000	77	77	
1990	Mar	16	10:00	1000	78	78	
1990	Mar	17	10:00	1000	79	79	
1990	Mar	18	10:00	1000	80	80	
1990	Mar	19	10:00	1000	81	81	
1990	Mar	20	10:00	1000	82	82	
1990	Mar	21	10:00	1000	83	83	
1990	Mar	22	10:00	1000	84	84	
1990	Mar	23	10:00	1000	85	85	
1990	Mar	24	10:00	1000	86	86	
1990	Mar	25	10:00	1000	87	87	
1990	Mar	26	10:00	1000	88	88	
1990	Mar	27	10:00	1000	89	89	
1990	Mar	28	10:00	1000	90	90	
1990	Mar	29	10:00	1000	91	91	
1990	Mar	30	10:00	1000	92	92	
1990	Mar	31	10:00	1000	93	93	
1990	Apr	1	10:00	1000	94	94	
1990	Apr	2	10:00	1000	95	95	
1990	Apr	3	10:00	1000	96	96	
1990	Apr	4	10:00	1000	97	97	
1990	Apr	5	10:00	1000	98	98	
1990	Apr	6	10:00	1000	99	99	
1990	Apr	7	10:00	1000	100	100	
1990	Apr	8	10:00	1000	101	101	
1990	Apr	9	10:00	1000	102	102	
1990	Apr	10	10:00	1000	103	103	
1990	Apr	11	10:00	1000	104	104	
1990	Apr	12	10:00	1000	105	105	
1990	Apr	13	10:00	1000	106	106	
1990	Apr	14	10:00	1000	107	107	
1990	Apr	15	10:00	1000	108	108	
1990	Apr	16	10:00	1000	109	109	
1990	Apr	17	10:00	1000	110	110	
1990	Apr	18	10:00	1000	111	111	
1990	Apr	19	10:00	1000	112	112	
1990	Apr	20	10:00	1000	113	113	
1990	Apr	21	10:00	1000	114	114	
1990	Apr	22	10:00	1000	115	115	
1990	Apr	23	10:00	1000	116	116	
1990	Apr	24	10:00	1000	117	117	
1990	Apr	25	10:00	1000	118	118	
1990	Apr	26	10:00	1000	119	119	
1990	Apr	27	10:00	1000	120	120	
1990	Apr	28	10:00	1000	121	121	
1990	Apr	29	10:00	1000	122	122	
1990	Apr	30	10:00	1000	123	123	
1990	Apr	31	10:00	1000	124	124	
1990	May	1	10:00	1000	125	125	
1990	May	2	10:00	1000	126	126	
1990	May	3	10:00	1000	127	127	
1990	May	4	10:00	1000	128	128	
1990	May	5	10:00	1000	129	129	
1990	May	6	10:00	1000	130	130	
1990	May	7	10:00	1000	131	131	
1990	May	8	10:00	1000	132	132	
1990	May	9	10:00	1000	133	133	
1990	May	10	10:00	1000	134	134	
1990	May	11	10:00	1000	135	135	
1990	May	12	10:00	1000	136	136	
1990	May	13	10:00	1000	137	137	
1990	May	14	10:00	1000	138	138	
1990	May	15	10:00	1000	139	139	
1990	May	16	10:00	1000	140	140	
1990	May	17	10:00	1000	141	141	
1990	May	18	10:00	1000	142	142	
1990	May	19	10:00	1000	143	143	
1990	May	20	10:00	1000	144	144	
1990	May	21	10:00	1000	145	145	
1990	May	22	10:00	1000	146	146	
1990	May	23	10:00	1000	147	147	
1990	May	24	10:00	1000	148	148	
1990	May	25	10:00	1000	149	149	
1990	May	26	10:00	1000	150	150	
1990	May	27	10:00	1000	151	151	
1990	May	28	10:00	1000	152	152	
1990	May	29	10:00	1000	153	153	
1990	May	30	10:00	1000	154	154	
1990	May	31	10:00	1000	155	155	
1990	Jun	1	10:00	1000	156	156	
1990	Jun	2	10:00	1000	157	157	
1990	Jun	3	10:00	1000	158	158	
1990	Jun	4	10:00	1000	159	159	
1990	Jun	5	10:00	1000	160	160	
1990	Jun	6	10:00	1000	161	161	
1990	Jun	7	10:00	1000	162	162	
1990	Jun	8	10:00	1000	163	163	
1990	Jun	9	10:00	1000	164	164	
1990	Jun	10	10:00	1000	165	165	
1990	Jun	11	10:00	1000	166	166	
1990	Jun	12	10:00	1000	167	167	
1990	Jun	13	10:00	1000	168	168	
1990	Jun	14	10:00	1000	169	169	
1990	Jun	15	10:00	1000	170	170	
1990	Jun	16	10:00	1000	171	171	
1990	Jun	17	10:00	1000	172	172	
1990	Jun	18	10:00	1000	173	173	
1990	Jun	19	10:00	1000	174	174	
1990	Jun	20	10:00	1000	175	175	
1990	Jun	21	10:00	1000	176	176	
1990	Jun	22	10:00	1000	177	177	
1990	Jun	23	10:00	1000	178	178	
1990	Jun	24	10:00	1000	179	179	
1990	Jun	25	10:00	1000	180	180	
1990	Jun	26	10:00	1000	181	181	
1990	Jun	27	10:00	1000	182	182	
1990	Jun	28	10:00	1000	183	183	
1990	Jun	29	10:00	1000	184	184	
1990	Jun	30	10:00	1000	185	185	
1990	Jun	31	10:00	1000	186	186	
1990	Jul	1	10:00	1000	187	187	
1990	Jul	2	10:00	1000	188	188	
1990	Jul	3	10:00	1000	189	189	
1990	Jul	4	10:00				

TABLE 9

Monilia bonordeni (After membrane passage)

Number of Egg	Culture from Egg	Growth			Time (Days)	State of Embryo	Growth Recovered	Microscopic Sections
		48 Hours	120 Hours	Final				
115	77	+	++	+	4	D	Yes	Yes
116	77	+		+	2	D	Yes	Yes
117	75	++		++	4	D	Yes	Yes
118	75	++	R	+	10	D	Yes	Yes
119	80	++		++	4	D	Yes	Yes
120	80	++		++	10	A	Yes	Yes
121	78	++		++	2	D	Yes	Yes
122	78	++		+++	10	D	Yes	Yes
182	81	++		++	4	D	Yes	No
183	81	++		++	4	D	Yes	No
184	81	++		++	2	D	Yes	No
185	81	++		++	4	D	Yes	No
186	81	++		++	2	D	Yes	No

# TABLE I Summary of the results of the experiments

Experiment No.	Time (min)	Temp. (°C)	Pressure (mm Hg)	Volume (ml)	Weight (g)	Calculated	Found
1	10	25	760	10	0.17	0.17	0.17
2	20	25	760	20	0.34	0.34	0.34
3	30	25	760	30	0.51	0.51	0.51
4	40	25	760	40	0.68	0.68	0.68
5	50	25	760	50	0.85	0.85	0.85
6	60	25	760	60	1.02	1.02	1.02
7	70	25	760	70	1.19	1.19	1.19
8	80	25	760	80	1.36	1.36	1.36
9	90	25	760	90	1.53	1.53	1.53
10	100	25	760	100	1.70	1.70	1.70
11	110	25	760	110	1.87	1.87	1.87
12	120	25	760	120	2.04	2.04	2.04
13	130	25	760	130	2.21	2.21	2.21
14	140	25	760	140	2.38	2.38	2.38
15	150	25	760	150	2.55	2.55	2.55
16	160	25	760	160	2.72	2.72	2.72
17	170	25	760	170	2.89	2.89	2.89
18	180	25	760	180	3.06	3.06	3.06
19	190	25	760	190	3.23	3.23	3.23
20	200	25	760	200	3.40	3.40	3.40



TABLE 10

Differentiation of Monilias Used with Sugar Fermentation \*

After 3 days		Species	Dextrin	Dextrose	Galactose	Inulin	Lactose	Levulose	Maltose	Mannose	Saccharose
		M. albicans, Strain 1	-	A-sl G-20	A-sl	-	-	A G-bubbles	A	A-sl G-20	A-sl
		M. albicans, Strain 2	-	A-sl G-60	A-sl	-	-	A G-100	A G-100	A-sl G-50	A-sl
		M. albicans, Strain 3	A-sl	A-very sl G-50	-	-	-	A G-100	A G-66	A G-30	A-sl G-50
		M. krusei	-	A-sl G-60	-	-	-	A G-25	-	A-sl G-50	-
		M. bonordeni	-	A-sl G-60	A-sl G-bubbles	-	-	A G-100	A G-15	A-sl G-50	A-sl G-60
		After 6 days									
		M. albicans, Strain 1	-	A G	A-sl	-	-	A G-10	A G-bubbles	A G-10	A-sl
		M. albicans, Strain 2	-	A G	A-sl	-	-	A G-80	A G-75	A-sl G-25	A-sl
		M. albicans, Strain 3	-	A G	-	-	-	A G-80	A G-75	A G-10	A G-15
		M. krusei	-	A G	-	-	-	A G-100	-	A-sl G-33	A-sl G-3
		M. bonordeni	-	A G	A-sl G-10	-	-	A G-80	A G-20	A G-40	A-sl G-33

\* Adapted from a Practical Classification of the Monilias by Martin, Jones, Yao and Lee (J. Bact., 1937, 34, 110-111).



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I am glad to hear that you are well and hope  
you are enjoying your vacation. I am well and  
hope you are enjoying your vacation.

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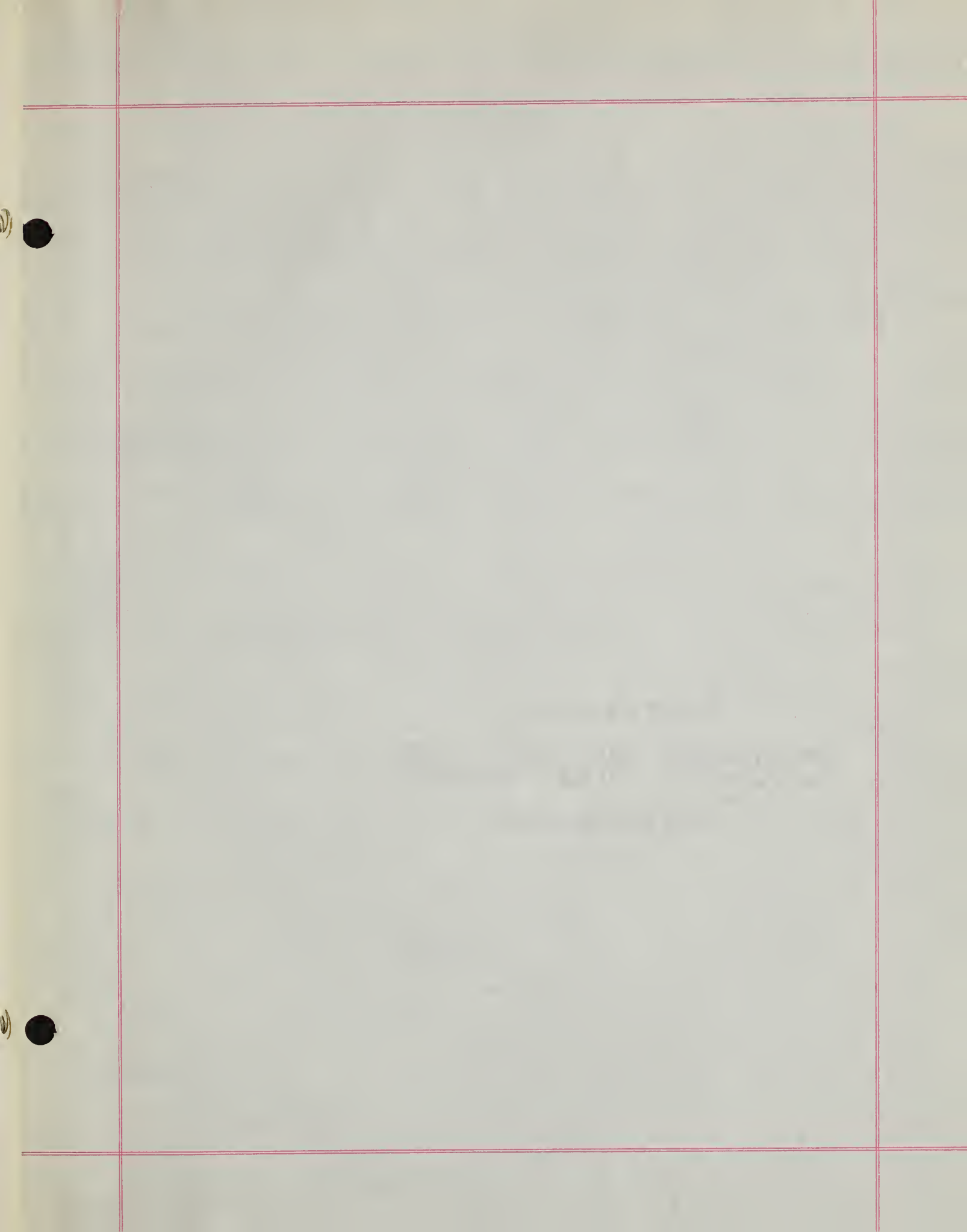
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