ESSAYS ON MYCOLOGY

(Contribution to the study of Fungi in Bello-Horizonte),

by

OCTAVIO DE MAGALHÃES and AROEIRA NEVES

[January 1912–June 1920],

(With Plates 56–91.).

This paper is meant to be a slight contribution to the study of the local mycological parasitology.

In it there will be found merely the essential for the understanding of our intention.

We shall exclude unessentials, minute details.

Cases will be brought up only in number sufficient for an ample illustration of our work.

Here we might repeat what a great physician said at the beginning of a profound book: «we are prevailed upon by no other thought than the desire to disclose the truth; in the search for truth we have been here as sincere as mayhap in the defense of some involuntary error».

FUNGI FOUND. MATERIAL AND METHODS OF WORK.

The material used for study was obtained from the Santa Casa Hospital and from private practice. The material from the lesions was of the most varied description. There is no call to specify here (Cf. further on). What we have to acknowledge is the courtesy of Professor ALEIXO, who was ever ready to give us the best chances for putting our program into execution.
### FUNGI FOUND

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### Notes

N. B. Fungi marked with an (*) will be studied separately in a paper published by one of us elsewhere.

(***) Considered as Ascomycetes

(*** ) About 50 new ones are not included, belonging to various species found later.
## Material Studied and Methods Employed

### 1) Direct Examination

<table>
<thead>
<tr>
<th>Unstained</th>
<th>Treated with lactophenol</th>
<th>Treated with hot potash (40% solution)</th>
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<td>Lactophenol-Coton blue</td>
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<td>Coton blue</td>
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<td>Iron-hæmatoxylin</td>
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<td>Hæmatoxylin-eosin</td>
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### 2) Inoculation into Culture-media

- Maltose-Sabouraud
- Glucose-Sabouraud
- Sabouraud with unrefined sugar (molasses)
- Glycerin-agar
- Ordinary-Agar
- Medium of Gorodkowa
- Dry slides
- Potato
- Banana
- Carrot
- Hanging drop with different culture media
Imbedding and staining of some of these cultures. Macroscopical and microscopical examination of the cultures.

3) Experimental Inoculation
   Rats
   Guinea-pigs
   Rabbits
   Monkeys
   Cats
   Man

   Different sites of inoculation

4) Examination of tissues obtained by biopsy
   Fixation
   10 % formaldehyde solution
   Sublimate-alcohol (Shaudinn)
   Giemsa
   Iron-haematoxylin
   Haematoxylin-eosin
   -Van Gieson

5) Drawings and measurements
   Zeiss Camera lucida
   Preparations, vital, stained, permanent and otherwise.

6) Biological tests
   Agglutination of spores
   Complement-fixation tests
   Intra-dermo reactions (Cuti-reactions) with fungi

We omitted, in this 6th. part, very purposely, the agglutination tests with the whole fungi. They have not shown up well in mycology. At any rate they have not been endorsed as similar reactions with bacteria have.

The discovery of GRUBER and DURHAM, except for isolated cases (1) is not yet an asset in diagnosis in the practice of present day mycology.

In the present paper, it must be stated, we have followed the plan drawn up by one of the authors and which is described in the thesis for a doctor's degree of Dr. MARIO COSTA (1919) (2).

ENDOMYCES ALBICANS

This commonly isolated fungus is often found in the saliva of children.

The culture is characteristic. To the unaided eye it mostly resembles colonies of yeasts. At other times it has a varying appearance. Wrinkling of the surface of the culture is however always very little marked.

It is not a common occurrence to find E. albicans and O. brasiliense associated in the same sputum. We have never found positive clinical evidence (except one case of a lesion of the mouth) of lesions caused by E. albicans. The biology of this fungus is the most characteristic.

VUILLEMIN's work, placing this species amongst the Ascomycetes, family Endomycetinae, allows of some doubts as to the unity of Endomyces.

We might point out that it is difficult to place in evidence ascii in the cultures of E. albicans. Only on one occasion, while examining cultures on a "dry slide", where we able to find an ascus of one type of E. albicans,
in spite of the fact that we had already tried a great number of poor culture-media with this end in view.

This would conduce us to the belief either that reproduction by asci is the rarest mode of reproduction of Endomyces or else that there are several types of Endomyces (1).

Some writers claim that inoculation of this fungus on culture-media containing creosote, makes it easily distinguishable from other fungi, as no growth would obtain.

We may state however that we have had luxuriant growths of *E. albicans* in culture-media with a small amount of that substance.

This fact we have mentioned in the paper on *O. brasiliense*, published in the Memorias do Instituto OSWALDO CRUZ.

One of the best means of distinguishing it is doubtless inoculation on alkaline culture-media, in which it does not develops, and on acid media on which it develops abundantly.

An interesting point about the cultures is that rod-shaped forms are also found.

Besides the culture-media employed up to now for this fungus, there is one yet to be recommended, simple and inexpensive—the banana (*Musa paradisiaca*). Fine-looking and luxurient growths are obtained on the surface of the fruit.

It is necessary to prepare this fruit-medium when the fruit has reached an optimum stage of ripeness and is neither green nor overripe. The medium is prepared in the same way as potato-medium. The latter have been employed even in this country with some success. (2)

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ALDO CASTELLANI:  — La Presse Médicale, 5 juillet 1917, n° 37, pag. 377. Note sur la Broncho-Spirochétose et les bronchites mycosiques.

ALDO CASTELLANI:  — Centralbl. f. Bakteriologie... B. 58, p. 236.

ALDO CASTELLANI:  — Further Observations on the fungi of the genus Endomyces found in Man. Arch. de Parasitologie, T. XVI. n° 2, 10 Julho 1913, pag. 184.


(1) On this subject, CASTELLANI has written numerous papers.
(2) Dr. PIRAJA DA SILVA. Duas novas especies de fungos productores de maduromycose no Brasil Brasil-Medico March 15, 1918 n, 81.
OIDIUM BRASILIENSE.

We wish to insist here upon the diagnosis of this fungus. One of the authors (1) has already written about it in a preliminary note. The process for the isolation of this fungus may serve as a model for the isolation of many other (filamentous) fungi from the same material.

The isolation from the sputum is done by the process of the «dry slides» (2), in the following manner:

The sputum lifted at the most gummy part with a previously heated platinum-loop, is allowed to slide about half way up the slide which is going to be inoculated. Running by its weight or rubbed along with a delicate pressure it finally flows into the liquid or paste which limits off the surface of the slide about a finger's width from the end.

Forty eight hours later, on the slide, along the parts contaminated, there are seen arising microscopic points which careful examination shows to be stellate. Under low magnification, these points reveal a dense dark centre with short and delicate strands radiating from it. This is a faithful reproduction of what is seen in the case of the Sporotrichum species (4).

One of these colonies is reinoculated into maltose—Sabouraud. In this medium small colonies are frequently seen to appear (after 48 hours). These colonies are rounded with a slightly prominent center and an irregular or smooth surface of a dark-brown colour and are surrounded by a crown of very delicate spicules.

These spicules are more easily seen when looked at slantingly. After growth an appearance is shown similar to that seen in Fig. 1. All bacterial contamination is thus easily obviated.

On the other hand, on a single slide all the botanical characters of the fungus may be seen together. We give phot. 51 and 52, obtained from inoculation on dry slides and which furnish new aspects of the evolution of O. brasiliense.

We had already found in preparations of O. brasilienense the «resistant cell», a type of large cell's which WEIL described in some yeasts. In this we find an aspect which discloses the origin of the rod-shaped elements of the fung. This is just a large cell, which after the formation inside it of these bacillary bodies, by successive division of chromatin and plasma, ruptures and and frees the simple products of division. These begin anew the cycle of the fungus. We have here a real vegetation-cyst.

In the cultures of O. brasiliense we were able to follow the evolution of these cyst-cell up to dehiscence.

At the beginning these cells are large, with the protoplasm uniform and staining deeply with GIELM SA. At this period no sign of division is to be made out within them. As they evolve, however, there is a grouping of chromatin—masses and round these elongate bodies of protoplasm. Within a short while the whole plasm of the cell is broken up by scissiparity and large cells are to be seen full of rod-shaped bodies, well stained by GIELM SA and finally there comes the stage of dehiscence.

Once free, the bodies again take up the cycle, mycelium, conidia and

(1) O. MAGALHÃES: Brasil-Medico, n. 24, July 15, 1918. XXXII. 185.
(3—4). Cf. Photos. 48 and 49.

(1) See also photographs 48 and 49.
so forth. Recently THEOBALD SMITH (1) described a fungus, very polymorphic in appearance, which was the cause of an epizootic pneumonia of young calves.

Besides the rod-shaped forms others have been described like cocci and like actinomycotic conglomerates. But in the whole article there is something indefinite which might perplex any novice in the field of arrhizophtylic forms.

SPOROTRICHOSIS

Cases of sporotrichosis are, together with those in which Oidium and Microsporum are found, the cases seen in the largest proportion in this work. Most of the clinical cases (about 31) were of lymphangitic lesions in which the initial sores caused by Sporotrichum were still to be seen (See Cases 1, 2 etc.).

An interesting story was that of a boy, in whom the initial lesion on the cheek, remained for long isolated, gummatous and purulent.

There was an exceedingly slight involvement of the lymph system of the neck and of the sub-maxillary region.

The enormous and indelible scar ensued when the necrotic tissue had been discharged and after treatment with iodids.

We also saw a lymph-gland involvement in the neck. The patient was brought to consultation as a possible case of Hodgkin's disease.

In smears made from the material obtained by puncture, fungus forms were found.

Sabouraud's culture-media with maltose gave, when inoculated, pure cultures fairly rich in pigment.

In this case there was no initial sore in the infection. Very likely, taking into account the seat of the lymph-gland infection, penetration had taken place through the mucosa of the mouth or through the tonsils.

It is a known fact that Sporotrichum forms may be found in the mouth without any diseased condition of it. It is not therefore extravagant to suppose that the fungus may have entered through some effraction of the mucous membrane next to the dental arches or at some other point.

This would be by no means the first instance of this kind in the literature on sporotrichosis.

BEURMANN and GOUGEROT's case VI is a very demonstrative one. (1)

In this case only the lack of a lesion, at any rate of a lesion visible to the naked eye was to be observed.

As far as we are informed, only of late has it appeared to be proved that they may find their way in through the normal mucous membranes without leaving any traces.

One of us, however, demonstrated in experimental work on monkeys this interesting and far-reaching property of the fungus. Oidium brasiliense.

For many parasitic diseases of unknown origin this might be the means of penetration in man or in animals.

This would be no exception in the chapter of organisms pathogenic as regards to man.

Another localisation, interesting because it was primary and because of the possible confusion with tuberculosis, was that seen in Phot. 11.

We saw another case very similar in its clinical appearance and in its response to treatment, as well as in the possible diagnostic confusion it might give rise to. In this second case the appea-

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rance of the lesion was clearly verrucous. The first was seen partly in epidermis and partly on mucous membrane (of nose), the second was purely epidermal.

There is another point which merits the attention of workers. It is that concerning the more or less obscure question of the incubation period. Almost all patients can be made to describe the mode of inoculation. They will thus incriminate a thorn or some other traumatic cause as being the source of the trouble.

In most cases, however, the question of term of incubation remains more or less obscure.

The case of our patient n. 1, with lymphangitic lesions, is however very patent in this respect. Forty eight hours after an insignificant and bloodless excoriation on a rose-thorn, the first symptoms of the condition put in their appearance. These were of pain locally at the part affected, which did not prevent him from working.

These symptoms of pain preceded the objective symptoms which led the patient to the Hospital, i.e. local ulceration and lymphangitic nodules.

In almost all cases it was possible to see the initial sporotricum sore.

Laboratory research undertaken on the material taken from patients may be mentioned here.

One particularity may be pointed out from the outset: it is that of the great poverty in fungus forms usually met with in material from human cases of infections (1).

Our attention was once called, in a tone of censure, to the fact that preparations seen in books and made abroad by authorities contained the fungi in enormous numbers. Well as a matter of fact, in the most careful and accurate investigation, in our laboratory, of 31 cases, only in 3 instances were the fungus elements plentiful in preparations. Negative results in direct examination were frequent.

The classic forms are also rare. Round forms, small and ovoid forms, with or without a septum are often met with.

Fixation by ordinary alcohol, heated, or by methylalcohol, and the GIE-SA stain, were generally sufficient for the first examination. Inoculation in culture media of the material was not dispensed with. This was also the decisive factor.

These cultures were made on «dry slides», on maltose-Sabouraud, on glucose-Sabouraud or on Sabouraud with moasses.

Cultures of sporotrichum on the first and second of these media are not pathognomonic for the fungus. Already one of us, in an article in the Brasil-Medico of June 15, 1918, has dealt with the question exhaustively.

Identical cultures are obtained with «Oidium brasilense», Endomyces albicans, Achorium schoenleinii, Penicillium glaucum, Monilia forms etc. (Cf. fig. 1).

We stated that: The macroscopic appearance, in «dry slides» and on maltose Sabouraud, were faithful reproductions of that obtained with Sporotrichum forms. It is a particularity on which we would like to direct the attention of workers. On page 565 (op. cit.) BEURMANN and GOUGEROT state:

«Il faut bien remarquer que ce contrôle microscopique est inutile lorsque l'on a suivi la technique que nous avons réglée et que l'on fait couramment le diagnostic bactériologique de sporotrichose, sans étuve et sans microscope, avec un simple tube de gélose glycosée loin de tout laboratoire». And further on page 562:

«Ce n'est donc que dans des cas

[1] See Cases 2, 3 etc.
exceptionnels que, le contrôle microscopique des cultures est nécessaire; c'est lorsque les cultures ont été faites sur des milieux défavorables ou sur des géloses glycosées peptonées brûlées ou trop vieilles et déséchées.

In the article of the same authors in KOLLE and WASSERMANN's Microbiology (1913), in GILBERT and BROUARDDEL's treatise (1910) in the book. «Les nouvelles mycoses», (Encyclopédie scientifique des aide-mémoire—Direct. LIAUTE'), in the book written by DOPTER and SACQUEPEÉE (Bactériologie, 1914), in that of AGASSE-LAFONT, in that of LUSTIG (1913), in fact in most works on the subject the same statements are found.

We obtained exactly the same cultures observed with the naked eye with the process of «dry slides» (phot. n.) as on maltose Sabouraud itself (Drawing n. 1) with Oidium brasiliense and with Endomyces albicans.

In the latter the resemblance was not limited to observation with the naked eye (phot. n. 50).

Examining cultures of Endomyces albicans just beginning to grow, we were able to find dispositions looking like pictures of what is supposed to be characteristic in Sporotrichum growths (1). Only posterior examination with the unaided eye and with the microscope can dispel the illusion. We will say nothing of certain free-living fungi and of Hemispora stellata. The latter has already been mentioned by DE BEURMANN and GOUGEROT as a possible cause of error, though one that is easily obviated.

It is important not to overlook, and we have a good deal of experience in this particular, that Sporotrichum forms do not always give typical cultures. Pleomorphism, to use a generally accepted term, is not infrequent among them.

What is not to be mistaken in smear-preparations and in «dry slides» is the disposition classical in the family of the Sporotrichaceae, sub-order Conidiosporata, genus Sporotrichum VUILLEMIN (1). This is evident. The picture botanically is always the same. (2)

Anomalous forms are rare. In the work of GOUGEROT cited we find already mentioned these curious aspects of the parasite. Macroscopical appearance is varied and uncertain. The question of pigmentation, for instance, has been thoroughly investigated by DAVID J. DAVIS (3).

He has proved that the question of pigmentation in the Sporotrichum cannot be made use of at all as a basis of classification. We feel that this demonstration of the fallibility of pigmentation as a criterion for classification in the genus Sporotrichum might soon be extended to a great number of known fungi.

We may, with a few experiments, say that we not only corroborate the evidence of DAVIS but still 'further assert that the macroscopical appearance of cultures is fallacious as a criterion. Cultures of classic appearance are seen in Photos. 3, 4, 2a. and 5. Others more with the appearance of yeasts (Phot. n. 5).

Langeron wishes to include in this group Sporotricha and Binocladiina [which were one Sporotricha].


There are cultures completely covered with very fine hairs (velvety appearance).

With further development this appearance comes to an end.

We obtained one specimen from a culture with the classic characters, which with time transformed itself in a type very similar to that of *Proteomyces infestans*. In this case resemblance was not only macroscopical. This was, in fact, the only instance we have met up to now of a botanical anomaly of the Sporotricha. This anomaly comprised the thickening of the mycelium, the form of the spores, and even in some points the stipital insertion of the latter. This anomaly, which in the opinion of many people would be enough for the creation of a new species, must be paralleled with that other anomaly described by DE BEURMANN and GOUGEROT in which a blastomycotic form of sporotricha is seen (1).

Another inconstant appearance nevertheless worthy of mentioning is that seen in phot. 6, in which the culture of the fungus, literally covered with a white powder, reminds one of the growths of certain blastosporous fungi (DE BEURMANN and GOUGEROT, op. cit., page 93, ascribe this appearance to pleomorphism).

This appearance is due to real aerial hyphae made up of mycelia placed side and of characteristic conidia. This appearance may or may not be continued with repeated reinoculation of media.

There are cultures which, from being pigmented, transform themselves into pigment-free growths after repeated reinoculation of culture-media. The converse is also seen.

There are parasitic forms which are somewhat uncommon.

These are minute cocci, with or without a double-outline, and, above all the rod-shaped forms. The latter, we were able to see in great numbers in a case of sporotrichosis typical both from the clinical and the microbiologic point of view.

These were for a long time passed over in silence or interpreted as degenerated or anomalous forms (1) (no mention being made of the Discomyces).

Rod-shaped forms have already been seen in this country with some degree of frequency in *Endomyces albicans*, *Oidium brasiliense*, *Achorium schoenleini*, Blastosporous forms, and, on a large scale, in the case of *Adenomyces cruzi* (O. MAGALHAES, A. NEVES, E. DIAS).

They are rods mostly short and stumpy with or without varied granulations. The origin of these forms was ascertained by one of the writers in the case of *Oidium brasiliense* (Photos. 51 and 52).

They are akin to those other small coccus forms of double outline, described in a type of cryptocoecus by G. VIANNA (2) and by north-american writers (3) (4) and which HARTMANN took to be a new protozoon. As in cryptocoecic, these forms arise from a big rounded cell, whose dehiscence is operated by a tear running at an acute angle, and setting free the rod-shaped bodies. The latter are formed by the scissiparity of the nucleo-protoplasmatic mass of the mother-cell, which may be considered a real cyst.

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In the field of serum-agglutinations for sporotrichum, which we often undertook, the excellence of spore-agglutination as compared with complement-fixation for sporotrichum should be pointed out.

The latter may fail when the former is positive.

As regards treatment, it may be mentioned, that the ministration of iodids gave capital results in 23 cases. In one case there was evident resistance, another got well spontaneously. Coexistence of syphilis is frequent in patients with sporotrichosis, as was shown by various means, including the WASSERMANN reaction.

We must mention here that cultures of Sporotricha, as also cultures of Achorium, imbedded and cut are of great value in the study of these parasites. Fixation must be carefully carried out—in Sublimate-alcohol—imbedding in paraffin and staining in haematoxylin with a good differentiation after it.

Inoculations of sporotricha in animals give mostly useful results. Rats gave us the best results in the study of this fungus. Nothing was observed in this chapter besides what BOUGE-ROT had already minutely described.

There is just one fact, similar to that observed in the human lesions, which ought to be pointed out, i.e. the scarcity of parasites in smears and sections of experimental sporotrichotic lesions.

In the comprehensive work of FRANCIS G. BLAKE on Sodoku and in others which followed on the same or allied subjects, there is always found the description of a fungus, which besides a most marked polymorphism displays numerous and constant rod-shaped forms.

As an anomaly of development in sporotricha, we found cultures covered with real spicula and reminding one of cultures of some blastosporous fungi.

CLINICAL CASES

Case of Sporotrichosis. Lymphangiatic form (corresponding to phot. n. 3).

M. T., Brazilian, brown, 18 years of age, domestic occupation. Family antecedents, parents dead, cause unknown. No brothers.

Personal antecedents. When she was a child the patient had measles and whooping-cough and later a slight attack of rheumatism which was rapidly overcome.

While at work, planting rose trees, she happened to wound herself very superficially, on the palmar surface of the right thumb.

Two days after this wound, which was insignificant, causing no bleeding and not preventing the patient from proceeding with her work, she began to feel very slight, but persistent, pains at the site of the wound.

Two months later there appeared a small ulcer at the same point, with lymph-gland swelling on the finger and on the outer and anterior surfaces of the right fore-arm; within a short time the axillary lymph-nodes were also involved.

Some of these lymph-nodes ulcerated as is to be seen from the photograph given; slight depressions of a perfectly circular shape resulted. No signs of a perfectly circular shape resulted. No sign of hereditary or acquired syphilis.

WASSERMANN Reaction: Negative.

Other organs perfectly normal.

Treatment:

Potassium iodid, 3 grams a day and local application of tincture of iodine. This treatment gave prompt results, and the patient was perfectly cured within a month.

Mycological Diagnosis: Sporotrichum beurmanni.

Pure culture with the classical cha-
acters. Inoculations in marmosets and other animals with positive results.

Bello Hórvizonte, September 1912.

J. M. S., a waiter, 23 years of age, single. Nothing of interest in anamnesis.

About two weeks ago he observed a pimple on the mesial border of the right arm, on the lower third. Later this pimple became ulcerated, which the patient attributed to repeated trauma.

As soon as the ulceration became more extensive, there appeared a little pain in the joint but not strong enough to cause any real discomfort.

At present there is to be seen an ulcer of irregular borders and showing at times small abscess which void a small quantity of pus when compressed. On the ground of the ulcer small vegetations of pinkish colour are to be seen.

On the anterior surface of the forearm, more or less at the middle of it, at the site of the initial sore, 6 gum mata were to be seen, the fourth being the most developed.

This lesions we punctured and with the material obtained inoculations in culture media and smear-preparations were made, with the advantage of using material from a shut lesion for these purposes. At the height of the fold of the elbow we could make out another little gumma, which was easily palpable.

On the arm, along the mesial border of the biceps, three more gum mata were to be seen, the lowest being the smallest. On the lower third, of the fore-arm between the ulnar border and the middle-line, in the vicinity of the initial sore, other gum mata are to be felt and seen, owing to the purplish colour which they present.

General state of patient good.
Direct examination: negative.
Cultural examination: positive.
Mycological diagnosis: Sporotrichum.

J. O. S., Portuguese, married. 27 of age, potter.

About 15 days ago, a small pimple or "nails head" as it is known popularly, about the size of a pin’s head, ulcerated and went on increasing progressively until it attained its present dimensions, which are of 1 centimeter and a few millimeters in antero-posterior diameter and of 0.5 cm. transversally, and located on the posterior surface of the left forearm.

The patient states that there had been no previous trauma.

At present there is the initial sore to be seen with the dimensions stated. Along the whole periphery can be clearly seen an infiltrated, inflammatory zone. The lesions is not painful and does not cause any discomfort to the patient. Nearer the mesial border a gumma is noticeable. Higher up, below the elbow and on the same border, three nodules, conspicuous on account of there red colour, are to be seen. Still higher up are another two nodules, of smaller dimensions, but still clearly perceptible and of the same colour. The general state of the patient is good. No fever. From the anamnesis is to be learnt that the patients mother always suffered from the liver. She had had no miscarriages. The patient’s father was always healthy.

Diagnosis: Sporotrichosis.
Treatment: Potassium Iodid.

Microscopical examination of the pus and serous fluid from the closed lesion: negative.

Cultural examination: positive.

Mycological diagnosis. Sporotrichum beurmanni.
Cured.

Sept. 19th., 1917.

dng at Capella Nova do Betim. Is vacci-
nated. Admitted Aug. 18th., 1917.

Anamnesis. A rare case of ocular
location, was studied with care by Prof.
LINNEU SILVA (see communication).

Family antecedents. Father alive,
age 55. Mother died 8 months ago with
a disease of throat. While alive she
had 9 children, of which 3 died at an
early age.

Personal antecedents. Whooping-
cough, while a child. Fracture of one of
the legs.

Study of present disease. About a
month ago, the patient noticed that a
small swelling had developed at the me-
sial angle of the left eye; soon after he
wounded the spot with his nail.

After this, the patient says that a
little below the mesial angle of the or-
bita, the skin became red and gave rise
to the formation of a phlyctena. Next
there developed three more swellings on
the left half of the face.

Present state. At the mesial angle,
there is to be seen on the left eye a
 verrucous crust which exudes a sero-
sanguinolent fluid. On the left half of
the face four other gumma are notice-
ced, two of which have already devel-
oped into pustules, whereas the other
two are still hard. Sub-maxillary lymph-
 nodes are involved on both sides. A
gumma very near the left sub-maxillary
lymph-node is full of pus the colour of
coffee with milk, as puncture showed. In
this part of the face an infiltration of
the course of the lymphatic vessels is
to be seen. Pur inoculated in the Sa-
bouraud culture-medium gave rise to
a pure culture of Sporotrichum beur-
manni.

Diagnosis: Sporotrichosis of the eye
(Dr. LINNEU SILVA).

Prognosis: mild.

Treatment: Potassium iodid.

Sporo-agglutination with the serum
of patient (JOLTRAIN's technic) stron-
gin positive at 1:30, weaA agglutination
at 1:50.

Complement-fixation test. Weakly
positive.

Mycological diagnosis: Sporotrichum
beurmanni.

Case which we owe to Prof. ALEI-
XO.

S. B. 5081. Name: ... Age: 19 years.
White, single, labourer. Resides at: Rio
acima. Vaccinated. Admitted Aug. 31th.,
1917.

Family antecedents. Father alive,
age 48. Mother died 12 years ago. She
had 5 children of which 1 died in its
first infancy.

Personal history. Patient had meas-
les. He was always weak. He has al-
ready suffered from diarrhoea.

History of the present disease. About
three months ago, the patient noticed
the appearance of a bleb on the mesial
surface of the left heel. This bleb was
broken open by the patient and dischar-
ged some lemon-yellow serosity. The
site of the bleb ulcerated soon after.

In the days following, new ulcers
formed along the right lower limb.
About a month ago he noticed that
the flexor group of muscles of the leg
went into a permanent contracture.

Present state. On the right the pa-
tient shows a chain of ulcerations, be-
inning at the mesial surface of the
right heel and running up almost to the
inguinal fold. The ulcerations vary in
size. Borders undermined, ground red
and irregular, discharging a thick yel-
low pus.

Diagnosis: Sporotrichosis of leg.

Prognosis: Favourable.

Direct examination. Very fine and
plentiful spindle-shaped forms are found
in all smears examined.

Cultures: positive.

Mycological diagnosis: Sporotrichum
beurmanni.

Two months ago there began a condition of the nose which produced pain and evil odour. She went to Prof. RENATO MACHADO for consultation and felt better, but the disease has extended to the nasal lobe, in the form of pimples which are itching and painful. Her brothers are healthy, as are also her parents. She has had intestinal worms. Nothing else was learnt from the patient.

Cervical and supra-epitrochlear lymph-nodes enlarged. The site of the lesion is slightly congested (active congestion). On this congested ground there arise a number of excrescences like warts, and varying in size from that of a pin’s head to that of a grain of rice. These warts, which are of soft consistence, are covered with a crust which, when removed, shows a damp but white surface (containing a very small amount of pus), very minutely mamillate. In the parts in which the excrescences are not seen there are scales covering the surface. The nasal mucous membrane continues affected, and mutatis mutandis the same lesions are to be seen.


MALASSEZIA FURFUR

The observations of this fungus are based on about 16 cases of Pityriasis versicolor.

We followed some of the processes indicated in the beginning of this work. It must be observed that we did not obtain even suggestion of growth on culture-media inoculated with material rich in fungi.

CASES OF PITYRIASIS VERSICOLOR.

Case 1.

S. B., student, single, residing in Bello-Horizonte. Squamous areas papular elevations with a geographic outline on chest and back mostly. Inoculations of Sabouraud culture-media negative. The maculae made the skin look as if burnt in certain areas. Lesions circinate, a pale pink, not soften covered with fine scales. Foci multiple, almost always small. There was confluence towards the posterior part of the trunk stiffly between the shoulder-blades.

Arms unaffected, especially at the lower part.

Neck also unaffected. At the zone of confluence and contrasting with the skin, the appearance of the spots is dark. The spots extend down to the abdomen, where the lesions are few and far between.

Evolution in 6 months. Pruritus moderate. (Sept. 9, 1917).

MYCOLOGICAL DIAGNOSIS: MALASSEZIA FURFUR.

Case 2.

J. C. L., 32 years of age, married, with one son of 14 months. Father and Mother dead. His mother died of a disease unknown to the patient; his father of a congestion of the brain. He has 13 grown-up brothers and sisters. Three brothers died, one during labour and the other two of tuberculosis.

The patient has had chicken-pox and measles. Five years ago he expelled a tape-worm. He has had no other parasitic disease. He does not own to having had syphilis.

Seven years ago he had hematuria and three years ago terebrant colics with intestinal perturbation. He sleeps well and has an excellent appetite. His
present disease began six years ago. It started on the chest. At present it extends over the whole chest, over the back and over the abdomen. The maculae are of the colour of coffee-with-milk and irregular. The appearance is typically that of pityriasis.

With baths the spots are supposed to have disappeared, according to the patient.

Preparations were made and cultures on Sabouraud, the latter negative.

Mycological diagnosis: Malassezia furfur.

Observation n. 3.

...... 26 years of age, laborer, white, residing in Curvello.

Parents healthy. He had 7 brothers; one of them died at 35, two are alive. He has had measles, influenza. The patient says that he has also had soft chancre, yaws, gonorrhoea and lymph-node infection. He also suffers from rheumatism and head-ache.

Of late the patient has been admitted to the Surgery Ward of Prof. BORGES DA COSTA and has undergone an operation for perforation of the palate of syphilitic origin.

Examination of the skin showed: on each side of the back, from the mesial angle of the scapula down to the 1st lumbar vertebra. Convergent lines are seen, which meet at the height of the first lumbar vertebra. Laterally the back is covered by one big area of darker pigmentation. In this little circular patches of skin unaffected by the disease in the middle of the space, comprised within the two lines mentioned above, 10 little patches were to be seen, which were conspicuous by their brown colour, contrasting with that of the normal skin. Above this space could be seen some confluence of patches and some parts unaffected by the parasite. On the front wall of the chest the same confluo-

of patches could also be seen covering almost all the skin.

On hands and arms patches were also found, confluent or isolated and the same may be said for the groin, buttocks and lower limbs.

The disease appeared in the patient about two years ago, soon after he had been given about eight injections of a mercury preparation. From that time they have augmented progressively and were not treated by the patient, since they caused no pain, although they provoked intense itching.

Case n. 4.

...... 19 years of age, single, laborer, Brazilian, from Mitozinhos.

Generalised tubercular leprosy. Ulnar nerves thickened, not sensitive. Eyebrows swollen. Microscopic examination of the nasal mucus showed bacilli with the characters of leprosy bacilli. Anesthesia of the lobe of the ear on the left side.

Anamnesis. Father died of heart failure. Mother healthy. Six children are alive and healthy. No other disease is remembered.

About six months ago there appeared macules on the neck. Which afterwards extended to chest, back and arms.

Whitish macules, conspicuously less pigmented than the neighbouring skin, are seen on the neck, especially on the rax, at the back, in an area comprised between the two posterior axillary lines and a little below the waist are seen some more depigmented macules of varying outline, often circular, the highest being bigger than the others. On the right side, and also on the back, there is one clear plaque of irregular outline and other smaller ones, ranging down to the size of a pin's head. On the right shoulder there is one more macule which begins at the back and
extends over to the anterior part, below the.

On the chest there is in front a macule of big size affecting almost the whole extension of a line drawn from one anterior axillary line (a vertical line passing through the front of the axilla) to the other. Below other smaller macules are seen.

On the arms similar but smaller and fewer plaques are seen. Fore-arms free. Scraping these whitish lesions a powder made up of scales from the last epidermal stratum is freed. With a fine pair of forceps it was possible to remove more extensive scales in which examination could be more efficiently carried out.

Mycological diagnosis: *Malassezia furfur*.

**EPIDERMOMYTON INGUINALE**

The cases which come under this heading in the final statistics are eight in number.

In four of these cultures of the fungus were obtained.

The failure of the remainder is due rather to the conditions of the patients than to anything unsatisfactory in the methods applied. It is caused by the age of the objective clinical symptoms.

Cases of two or three years standing do not as a rule permit cultivation of the fungus.

Besides this it has to be borne in mind that in nearly all the cases patients apply all sorts of treatment to their lesions so that, besides altering the clinical appearance of the lesions, they often affect the vitality of the parasite. An instance of this is the case showing localisation on the feet; this case, of two years standing, had all the macroscopic characters of inguinal epidermophytinfection, but did not permit cultivation of the fungus. Our case 3 indi-

cates a point to be emphasized, i.e., relapse in this dermal mycosis.

However small the residues of the disease, if it should not have been radically extirpated, the fungus will soon find an opportunity for developing anew in the same or in another part of the body.

The foci of infection must be very carefully extinguished, only in this manner can the disease be dealt with.

The relapses are often the cause of strange localisations of the fungus.

Not long ago RICHARD S. WEISS described in a memoir an unusual localisation of Epidermophytin (1).

**CASES OBSERVED**

Case n. 1. N. 1818. student, Aug. 24, 1917.

About 20 days ago the patient observed a slight desquamation at the bottom of the left cruro-scrotal fold, unaccompanied by pruritus. Slightly before he had treated himself against Pediculus pubis with mercurial ointment, so that he attributed it to the mercury the lesions of the cutaneous tegument of the fold. During the last week he has felt a good deal of itching. At the point indicated, an area, pale in the center and red round the edge, clearly bow-shaped, is to be seen. The epidermis of the central part of the area is practically restored while the periphery, specially within the red bow, shows typical micro-vesicles. No other lesion is observed elsewhere. It is seen that application of a 1% chloroform solution of iodised chrysanthen makes the vesication more apparent and it becomes evident that the whole red halo is vesicular; the same is seen almost in the middle of the lesion. The patient before

(1) RICHARD S. WEISS—The Journal of American Medical Association, n. 13, 1917 September, p. 19, V, LXIX.
consulting had already made use of nitrate of silver in a 2% aqueous solution, the only effect of which was to irritate the skin considerably so that the lesion became very conspicuous on account of the red colour it took.

Scales examined in lactophenol show almost immediately: plentiful mycelian filaments, septate, some of them with terminal and intercalar chlamydospores.

Treatment: Chloroformic solution of iodised chrysarobin 1%.

Clinical Diagnosis: Eczema marginatum of Hebra.

N. B. The lesions described are the ones of the crural fold. Those of the scrotal fold are almost invisible, only desquamation being noticeable.

Microscopic examination: positive.

Culture: Positive.

Mycological Diagnosis: Epidermophyton inguinale.

Case n. 2.

......., 24 years of age, Brazilian, Minas Geraes, residing at Hotel Globo, single, medical student. May 23rd, 1917, n. 1639.

More than a month ago the patient observed a small area on the upper part of the inner surface of the left thigh, not far from the crural fold. It extended eccentrically. On the symmetric part of the other thigh, there appeared ten days ago another area. No pruritus on the lesions of the leg, but on the scrotum, on the left side, where a new lesion is starting, there is considerable itching.

Clinical diagnosis: Eczema marginatum of Hebra.

Microscopic examination: positive.

Culture: Positive.

Mycological Diagnosis: Epidermophyton inguinale.

Case n. 3.

......., 24 years of age, brown, policeman. Admitted May 7, 1917, n. 1671.

Anamnesis. The patient has already come to consult for the same disease, more than a year ago. Inguino-cruro-scratal fold with very pruriginous patches. He improved after the first consultation, but was not cured and of late he has become much worse. Other places have since become contaminated. Status praesens: on the right inguino-cruro-scratal region, large erythematous-squamous patches, discretely vesicular round the edges. They extend to a considerable extent along the upper inner surface of the thigh. At this part a big inner part of a patch already cured is to be seen. A certain amount of serosity which has a smell, owing to fermentation, is secreted. Small and minute areolar patches in the vicinity. The left inguino-cruro-scratal patch is of tiny dimensions. Small patches disseminated on both glutal regions, very pruriginous, especially after the clothes are removed. On the balano-preputial groove, near the frenulum there is a certain amount of irritation. On the beard there are some patches, which are however discrete. He made one application of chrysarobin, only on the pudenda. This application, as was to be expected, irritated the lesions considerably. Besides the localisations already mentioned, numerous desquamative lesions of a very pruriginous nature are to be found between the toes on both feet.

Treatment: Zinci oxidi 4 gm.0.

Boricinae 4 gm.0.

Axungiae benzoatae 50 gm.0.

This after an application of the chloroformic solution of chrysarobin.

Clinical diagnosis: Eczema marginatum of Hebra.

 Cultures with material from the inguinal lesions negative; positive with scales taken from the lesions between the toes.

Mycological Diagnosis: Epidermophyton inguinale.
Case n. 4.

O. N. L., 22 years of age, white, residing in Minas Geraes, rua Carajóis, single, student, August 28, 1917, n. 1829.

Anamnesis. About 2 months ago the patient observed an eruption on the inguino-scrotal skin, at first slightly squamous and becoming red after a time which he attributed to his having applied soap, mercurial ointment and Helmerich's ointment. Pruritus extremely intense, specially at night. Objectively a red inflammatory patch, squamous and with its edges relatively little colored in comparison with the center, is to be seen. The scrotal patch is more squamous than that of the thigh.

On the side of the thigh of the cruso-scrotal fold is seen, a circinate lesion with edges more squamous, redder and slightly microvesicules, and, on the scrotal side, another circinate lesion with squamous productions. On the lesions of the left side no vesicles are seen, as can be proved with chloroform-iodine-chrysarobin. On both sides traumatic dermatitis, with small furuncles and follicular lesions.

Clinical Diagnosis: Eczema marginatum of Hebra.

Microscopical examination: positive. Mycological Diagnosis: Epidermophyton inguinale.

Case n. 5.

N. B., 19 years of age, white, Minas Geraes, residing at rua Sapucahy n. 445, single, employed in business, Feb. 20th, 1918, n. 2069.

About a year ago he observed the appearance of some very pruriginous patches on the left side of the thigh facing the scrotum. These patches progressed until they occupied the sites in which they are now seen.

Status praesens: On the left inguino-crural fold, a large patch with a non-colored center and festooned edges which are reddish and slightly scaly with adherent scales. A smaller patch on the right side. Pubic patches hidden under hair. Two circular patches on the left thigh. In none of these patches is there real vesiculation but only traces. Discrete secondary lesions of the skin on pubis. Gonorrhea. Left mesial inguinal lymph-node swollen and hard. No hard chancrre at present. He has already treated the patches with iodine.

Microscopical examination of scales: positive.

Clinical diagnosis: Eczema marginatum.

Mycological diagnosis: Epidermophyton inguinale.

Attention must be called to our observation n. 3, of which an illustration is given. (1).

Two other photographs are also reproduced corresponding to cases 1 and 4.

Of the ringworm groups, cultures of E. inguinale are those which most readily appear with a pleomorphic aspect.

Already SABOURAUD had mentioned what he rightly called lesions of old age. (2)

According to our way of thinking, this question of pleomorphism is really far more complex than is ordinarily thought. We may some day return to the point with more detail. At present we shall content ourselves with reproducing our photograph n. 4 (the 16th. of the general set of photographs) of a culture obtained by inoculation in a culture-medium of scales from the axilla.

Culture n. 1 (18 of the general series) is a growth of ten days; photo n. 2 (17th. of the general series) in an Er. lenmayer flask, in which is seen, in the common form of beginning pleomorphism spite of the great development of the culture.

Repeated reinoculation into appropriate culture-media may obviate this drawback. There are times when with four days growth nothing can be made out in the growths.

At first the culture is light-coloured, fluffy round the edges, and some umbilication is seen. Later the umbilication transforms itself into symmetric grooves looking like a maltese cross.

Subsequent growth of the fungus, however, modifies this aspect into yet another, i.e. that of real furrows or circumvallation without symmetry, in which often pleomorphic white fluffy flakes are appearing (Phot. n. 17 of the general series).

Peripheric irradiations of the growths reach, after some time, the internal borders of the flasks. Pleomorphism, which may be seen within the first new days of growth, may also show itself at the 22nd inoculation in culture-media of the fungus. SABOURAUD (1) describes the culture of *E. inguinalis* as having at all times a lemon-yellow colour.

The general and constant colour of the cultures with which we worked was a dirty white.

But we do not for that matter think ourselves entitled to a "new species."

Already in a former chapter, we have given our opinion on this question of pigmentation.

We have yet to mention direct examination of human material for diagnosis. The most important characteristic which dominates this question in that of the intense polymorphism of the fungus in human material.

This is yet a confirmation of what SABOURAUD (2) with his usual authority asserted when speaking of the species diagnosis and the differential diagnosis.

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(1) Ob. cit. pag. 442.
(2) Ob. cit. pag. 438-439.

TRICHOPHYTIA

We will next describe a case of trichophytia of the beard seen in the ward for skin and venereal diseases directed by Prof. ANTONIO ALEIXO. This was the first time a case of trichophyton-infection was seen in this city, at any rate by us. The case is as follows:

Case Report: C. C., 21 years of age, white, Italian, residing at Mata-douro, single, cartdriver, working for the local lard-packing plant. He was admitted May 5, 1917, and registered as n. 1608. The patient states that fifteen days ago he went to the barber and two days later noticed a round patch of 2 cm. of diameter and pruriginous. While this primary lesion was yet developing, the patient was noticing, about three days later, the appearance of fresh lesions. The primary patch was somewhat painful.

Symptoms now present: The patient shows a patch which measures 6 cm. (antero-posterior diameter) by 5 cm. (vertical diameter) near the right parotid region. This patch is of an erythematous-pustular nature and covered with crusts. Its shape reminds one of two confluent circles. Pustules and crusts are more numerous in the central part, in which there would seem to have been a recrudescence. The periphery is clearly outlined by an epidermis finely desquamated. The hairs show no macroscopical evidence of disease. No alopecia is seen in the vicinity. Only small and minute elevations, rather scaly than vesicular, are to be seen. Behind the ear a patch of a larger scale is seen. At the confines of nose and cheek a patch of about 2 cm. in diameter, approximately, and circinate, with a central area of normal appearance is seen. Lesions seen at edges are not continuous.
The principal patch, after the first application of X-rays, during 10 minutes, became elevated and gave evidence of a subcutaneous suppurrative process. There was discharge of a good deal of pultaceous material.

MICROSCOPICAL EXAMINATION

Microscopic examination of material taken from the lesions showed, in the hairs an ectothrix trichophyte with large spores, in rows not easily distinguished and making up almost the whole of the sheath in which the hairshaft was enveloped. The hairs, when pulled out, broke of fairly low down but brought with them the sheath which was whitish and thick.

There were spores of large dimensions and some rows of more or less regular spores. The hairs examined showed more or less the appearance seen on fig. 178, entitled "T. equinum MATRUCHOT autour du poil et dans le poil humain," of SABOURAUD's book, Les Téiges, Paris, 1910, page 250.

CULTURES

On Saboraud medium with malt-sugar, glycoce and with unrefined sugar (molasses), cultures resembling closely those of T. crebriforme, and, at other times T. asteroides, were obtained. On simple and on glycerin-potatoes a damp growth of yellow ochre were seen.

At present, on reinoculation of SABOURAUD's medium with glucose with material from one of our former cultures, a form of the fungus was obtained reminding one of T. effractus. This shows once more the uncertain nature of external appearance of the fungi and the difficulty of diagnosis with the unaided eye of cultures grown on the usual media.

Examination of cultures, i. e., of fragments removed and dissociated in lacto-phenol, showed us broad or medium mycelia seplate at short intervals, some of them looking like the racket-shaped mycelia of the microspora, intercalate and terminal chlamydomospores, conidia.

With the aid of the disposition of the parasit· on the hairs and its characteristic culture on potatoes it was possible to classify this parasite. It is certainly T. equinum MATRUCHOT et DAS-SONVILLE. This became still more evident after the following fact.

While still under treatment, the patient noticed certain lesions on the fur of the animals with which he worked.

We were fortunate enough to be able to examine de visu the animals with the disease.

The lesions were typical lesions of tinea tonsurans.

The barber could thus be exempted from the blame with which he had been shouldered owing to ignorance of the animal origin of the condition caused by the fungus.

To this report we may add a photograph of the culture of the parasite on glucose SABOURAUD. Nineteen days' growth, Phot. n. 1.

MICROSPORUM INFECTIONS

During the year of 1917 and some months of 1918 and during a like period of 1918 and 1919 we had the opportunity of seeing, besides cases of Sporotrichosis, Favus and some other mycoses, a few cases of Microsporon-infection.

But from the outset it will be well to point out the difficulties encountered at the beginning of our studies, i. e. our inability to obtain crude malt-sugar, so necessary to the growth of these fungi.

The material studied consisted not only of hair taken from the microscopic patches of tonsured, but also of scales taken from the erythematato-squamous
patches which marked the site of inoculation of the fungus on the skin.

For this examination we used mostly AMANN'S lactophenol which is an excellent fluid, we find, for this kind of examination. Staining was not often resorted to. When it was made use of, we carried it out according to BIZZOZERO and FIRKET'S technic. Besides this, caustic potash at 40% with subsequent heating was also applied.

AMANN'S lactophenol not only keeps the preparations very nicely, but also makes them quite clear and gives a very good differentiation of the fungus. It would be of great advantage, if a substance for framing the cover-glasses less soluble in the fluid could be found. The substance used by us, a mixture of bees wax and rosin, diffuses in the conserving-fluid and darkens the preparation, which becomes difficult or impossible to examine. This point should be a matter for further research. It ought not to be very difficult to avoid this drawback the prevention of which would seem to be already under way, with the use of a new mixture of lanolin and rosin, which ought to be less friable and diffusible than that of Krönig's.

Culture-media used were: Sabouraud with glucose and maltose, potato and Sabouraud's medium with unrefined molasses.

In the latter, however, pleomorphic forms are rather prone to appear, which are very discouraging for those who are studying these fungi.

Immediately after epilation we took care to examined the hairs.

The technic used was the following:
1. Epilation. A small forceps was used for epilation.

With this the hairs could be easily removed from the central part of the microsporon-lesion or patch and from the marginal parts or edges.

2. Next the hairs removed were placed between two glass slides; previously washed and sterilised in a spirit-flame. The hairs were left until examination was performed.

Scales were simply lifted off with the edge of a surgical knife and placed in the same way between slides pending examination.

The appearance of these hairs when pulled out with forceps or with the finger nails is very characteristic, and we need not go much into its description. For the latter the reader may be referred to SABOURAUD's description on pages 147 and 148 of his book (1). It was not always easy to obtain hair-shafts as the hairs are very brittle. When it is possible to get a hair, it has generally lost its bulb.

This is a not unknown fact and SABOURAUD describes it at great length in the book we have quoted.

As a precaution we placed in the same microscopic preparation several fragments of hair. In this manner it was possible to follow in one microscopic preparation the distribution of the parasite along the whole extension of the hair.

Once three or four pieces, entire or otherwise, of hair had been placed on the slides, a few drops of AMANN'S fluid were added. Next the preparation was slightly heated and after getting rid of most of the air-bubbles the preparation would be shut, as well as possible, under cover-glass.

The technic of preparations with potash, of staining and so forth is too well known for us to enter into the matter.

CASES SEEN

From February 1917 up to date (Oct. 1920), we have had the opportu-
nity of studying 24 cases of microspor-on-infestations which fall in the follow-
ing groups:

M. Lanosum......... 18
M. Audouini ........ 5
M. felineum......... 1

CULTURES

Cultures of this fungus exact very often the most elaborate work.

Not infrequently it is impossible to identify the microsporum by direct
examination alone.

When the organisms are found on parts without hair, the question must
generally be resolved by culture.

This is what we have tried to do, obtaining good results, in spite of the
difficulties already pointed out.

Inoculations of culture media were made into Erlenmeyer flasks and were
subjected to careful scrutiny every day.

From the report of these observation we may draw the following inferen-
ces:

1. The growth attains a size already worthy of attention from the third to
   the fourth day.

2. The maximum growth is reached between the fifteenth and twenty-fifth
day of the growth (and averages 6 cm. by 14).

These results were obtained on the
SABOURAUD’S malt-sugar culture-me-
dium (the classic medium). The same
thing is to be seen on SABOURAUD
with glucose or with unrefined molas-

Cultures of M. lanosum, inoculated
on the latter, do not show pleomorphic
forms, if case be taken to reinculcate
them every fortnight or at most every
twenty days.

Pigment-formation is not rare among
these fungi.

In M. lanosum occasionally a pink
pigmentation was seen and sometimes a
dirty white coloration.

In cultures of M. audouini, pigmen-
tation is at first grey and afterwards
yellowish brown, the colour of carrots.
This pigmentation is most characteristic
on potatoes.

However this character, as we can-
not repeat too often, is one of the most
inconstant in cultures, not only of hy-
phomycetes but of fungi in general.

Cultural pleomorphism is extremely
common in spite of most care to avoid
it.

Wherever possible every method
and every resource should be resorted
to in examining and classifying micro-
spora. Long experience with these fungi
has led us to this opinion.

When ever possible, also, the «clas-
ic» culture-media should be made use of.

As to the liveliness of growth, we
were not able to observe anything new.
It seemed to us that M. lanosum
developed just as quickly as M. audouini.
SABOURAUD’s experience which is
contradictory in this respect, must be
accounted for by different atmospheric
conditions (of heat and dampness, for
instance).

Here, in Belo-Horizonte, the tem-
perature does not vary much and os-
cillated very little during the time we
observed.

Oscillations are rather insignificant
when compared with those of the Old
World.

Hence, perhaps, temperature is of
slight influence in the growth of fungi,
although more important than altitude.

The influence that height might have on the development of fungi and
principally of parasitic fungi has al-
ready been investigated long ago. Re-
cently in a paper on phytopathology
J. DUFRENOY (1) studied comparatively

(1) J. DUFRENOY, Bull. Trimest. de la Société de
Mycologie de France XXXIV, 1918, 8.
with special detail the parasitic fungi in high places and on the plain.

Disease, i.e. parasitism, was found at all heights with the same intensity, provided that the host were present. This was the author's chief conclusion.

Greater, however, than atmospheric conditions or altitude, appears to be the influence of civilisation on certain parasitic fungi. Urban centres are poor in mycotic affections. Rural centres and country places have a great abundance of them.

This was shown strikingly by the experience gained during the Great War.

Subjects, up to then unattained by pathologic conditions due to fungi, became infected with a great number of fungi, after they had been exposed to the hard experience of life in the trenches, in constant contact with the soil (1, 2, 3).

Perhaps later we may have a chance to go into these matters.

Our cultures were always kept at the temperature of the surroundings; in no case was it necessary to make use of incubators (neither for microspora or for other fungi).

As to the morphologic appearance of cultures there is something to be added. Let us take M. audouini as an instance.

In the first days the culture on SABOURAUD's culture-media with malt-sugar is without any fluff, of a pure white, and, as it becomes older, the appears more compact and shorter, and less pure white, of a greyish white. At the same time as development takes place, three or four grooves divide the culture in a number of segments. As cultures grow older a new set of grooves appears between the former and divides the culture anew. This is the classic process in M. audouini. For greater clearness photographs of cases 1672, 1671 and 1730 are given. (Phot. 22, 23, 24).

As to the appearance of M. lanosum, nothing more need be said. The centre of the culture is umbilicated. The woolly ring forms a prominent elevation round this umbilication of a pure white colour which becomes yellowish, as the culture grows old. The periphery of the culture is formed by a set of irraditating grooves. Over the whole growth there is a delicate and grey fluff (on SABOURAUD's medium with malt-sugar). On the glucose medium the culture is also woolly, but irregularly so, not in a ring as on the maltose medium (cf. photos. 26, 27 and 28 which complete this description). In phot. 25 a pleomorphic form is to be seen.

MICROSCOPIC EXAMINATION OF CULTURES

This examination was carried out with the following conditions:

1) In a hanging drop, in Boettcher's glass cell;

2) In fragments of culture dissociated in AMANN's lactophenol;

3) After fixation of smears of cultures and colouring with GIEMSA or and Gram stains;

4) In the growth on the walls of tubes.

The fourth process can generally be used to substitute the first, which is more difficult to put into practice, with the care necessary to avoid contaminations. It is very easy to observe the disposition of hyphae in this manner. Nothing is easier than to place the tube in which the material has been inoculated under the objective of the

(2) E. ANTOINE, Annales de l'Institut Pasteur, Paris, Mai 1911, n. 5 page 202.
(3) RONDOER et PELLISSIER, Annales de l'Institut Pasteur, 1914, Nov.
microscope. Focalisation is not difficult.

Compensating oculars 8, 12 or 18 with objective B of ZEISS are of great value. In this manner we were able to study the sterile diverging filaments, the spindle-forms at their first stages or massive dilatations of more or less granular surface and rare. In the hanging drops we could observe a middle zone, bearing erect filaments of a slightly fusiform appearance at one extremity, a peripheral zone made up or radial filaments, which were creeping and sterile, and a central zone in which small erect hyphae could be seen arising from delicate, caducous, piriform conidia. Summing up we might say that in M. audouini grown in hanging drop, as in the description, given, we were able to observe nothing but what SABOURAUD had already seen. The same hyphomycete observed in the 2nd. condition with more powerful magnification (object. E, dry, and comp. oc. 4 or 8) showed us besides the spindle-shaped organs, full of granulations but not septate, the various forms of mycelium, i.e. the straight, the racket-shaped, spore-bearing hyphae with external conidia, with lateral ramifications and bifurcations and sterile, spore-bearing hyphae. There are also chlamydoспорes. Besides these, pectinate hyphae are clearly seen in these preparations.

In cultures of M. lanosum on hanging drop we saw the following: compact central part from which emerge numerous more or less straight, creeping mycelian filaments. This central part is made up of compact mycelium, bearing on slender and curved peduncles a great number of spindles easily seen with low power. With greater magnification, the appearance is more attractive with straight, septate mycelia, a great number of small and large spindles, like leaves and va.

rying in size and number of compartments loci. In fragments of culture dissociated in lactophenol, we observed besides these, intercalar and at times intercalar chlamydoспорes, and spore-bearing thyrsi.

In certain cultures, as for instance in case 1943, spore-bearing thyrsi were more plentiful than spindles, a fact in disagreement with SABOURAUD when he says: «Les fuseaux sont bien plus nombreux dans les cultures que les thyrses sporifères». (1)

We observed that certain spindles, when looked at from above, i.e. when their extremities are brought nearer, assume a spherical form, compressed at the poles resembling a blastomycotic cell, full of granulations, and with disappearance of the loci (Cf. preparations from the culture obtained from case B. P.).

LOCATION OF MICROSPORUM LESIONS.

Based on our observation we may classify them summarily under the following heads:

1. Lesions located on the scalp;
2. Lesions located on the skin;
3. Lesions located at the same time on scalp and skin or mixed location of lesions.

On beard and moustache we were unable to see a single case. The first type is the more commonly seen and is the microsporous tonsurans. Clinically we were unable to differentiate ring-worm caused by M. audouini from that caused by M. lanosum. The lesions consist of small patches, or of medium and at times large patches covered with greyish scales with a few or no healthy hairs left on its surface. When hair-shafts are found, they are

(1) Les Teignes, op. cit. page 678, 19010.
brittle, greyish, enveloped in a sheath. The appearance is characteristic.

In some cases the patches are accompanied by reddening and irritation of the neighbouring parts. Others showed a great number of small patches more or less spread over the scalp.

Cases in which irritation was an evident feature were those of long-standing.

In agreement with SABOURAUD, the evolution of these lesions may be divided in two periods. The first is characterised by the appearance of microsporuous patches and in the second there appear inflammatory manifestations. Microsporum patches do not grow indefinitely. When they get to a certain point they halt in their development and at most they attain a size of a circle an inch in diameter. In this part of the body it is rare to see only one patch; almost always there are two, three or more of them (See photographs n. 29, 30, 31 and 32 and case reports n. 1 and 1 A).

In view of the nature of this paper we need not go into the details of these lesions any further.

2. Location on the smooth skin. This location is extremely common here in Bello Horizonte. The lesions show themselves as erythematous-squamous patches, not or very little pruriginous, with orbicular, circular or at times circinate with several rings, limited by a reddened squamous edge, the centre slightly beset by adherent scales.

With this location on the smooth skin alone we can present the following cases, whose aetiology has been made sure by microscopic examination and culture.

CASE REPORTS

G. P., registered at the Policlinic on June 17, 1918, Brazilian, residing in Bello-Horizonte, school-girl (Case n. 2).

Family antecedents see case n. 3 of this patient’s sister.

As to the patient’s own history she mentions suffering from various intestinal affections. Five days ago she noticed a small patch on the left side of the neck which was almost cured. Afterwards on the same side, at the back of the neck another patch. Examination shows on the back of the neck an erythematous-squamous, pruriginous patch. Of the primary patch there are no traces left.

Diagnosis Trichophytia (?).

Mycological diagnosis: M. lanosum.

Case n. 3.

B. P. June 17, 1918, 7 years of age, white, Brazilian, residing in Bello Horizonte in the quarter of V. de S. Vincent, school-girl. Has had gonorrhoea and syphilis.

Personal history. Has had abscesses, inflammatory affections of the skin, intestinal affections.

History of the disease. Five days ago the patient’s mother noticed a lesion on the right shoulder and three on the patient’s legs. These lesions soon multiplied. In searching for the origin of these patches it is noteworthy that in the neighbourhood there lived a sick cat and in the same house another cat which already showed cutaneous lesions.

Present symptoms. On the left shoulder an orbicular patch of about 2 cm. in diameter and erythematous-squamous.

On the two thighs are also several conspicuous orbicular patches, encircled by erythematous-squamous edges. In some patches a central erythematous-squamous point is also to be seen. Pruritus inconsiderable.

Clinical diagnosis: Trichophytia (?).

Mycological diagnosis: M. lanosum.

In this and in the preceding cases the origin of the parasite is in all probability from an animal. In this case the fungi were obtained from cats, from
the one of the house or from the one of the neighbourhood. There was yet another patient from the vicinity, which we also had the opportunity to see and who showed exactly the same appearance as the ones described. Patients of cases 2 and 3 are sisters.

Case n. 4.

S. de P., one year and four months, white, Belo Horizonte, Barroca, November 14, 1917, n. 1946. A few days ago there appeared a lesion on the left arm. In the same house a man and a boy also had the same sort of thing. The baby does not report any itching.

On the dorsal surface of the fore-arm a circular patch 2 cm. in diameter, and progressing excentrically, is seen. The periphery is marked by vesicular productions and in the centre the skin is normal. The patch is perfectly circinate and shows itself as a reddish, slightly squamous and vesicular ring. Nothing was found elsewhere, not even on the head.

Clinical diagnosis: Trichophytia (?).

Mycological diagnosis: *M. lanosum.*

Case n. 5.

M. de C., one year and seven months. Brazilian, Belo Horizonte. About fifteen days ago there appeared a lesion on the face, with some itching. Next there broke out similar lesions on the chest and arms, causing a good deal of itching. He believes that his disease arose from that of a cat, which appeared at his place, very much emaciated and covered with patches of alopecia, on the face, especially on the right side, there are patches, some of them minute, pin-pricked and squamous. Other larger ones, with a squamous, circular edge surrounding a centre of nearly normal skin, which is more opaque. The largest are the size of a collar stud shirt-front button. Some of them are circinate with the circle incompletely closed. On the palmar surface of the elbow there is a papular efflorescence, about 5 cm. in size, with thickened edges and a depression.

On the right arm there are three patches. On the left there is one and a point. On the back a number of them are to be seen like acne. On the scalp there are none. No evidence of syphilis or of any other disease is found. There is no clear vesicle-formation.

Clinical diagnosis: Trichophytia (?).

Mycological Diagnosis: *M. lanosum.*

Case n. 6.

... 3 years old, white, Brazilian, residing in Belo Horizonte, Rua Curitiba 763. April 27, 1918, n. 2156.

Eight days ago there appeared on the child’s arm a pruriginous patch. This child was always playing with the little patient n. 2155 of the preceding case-report. It is not known however if contagion took place from human being to human being or from animal to human. This child also played with the cat already mentioned in case-report 5. The parents of this patient are healthy, as is also the patient. The child shows a very clear circular patch on the left arm.

The center of the patch is scaly, but without detached scales; it is opaqueness, pleated, limited by a circle of adherent scales, outside of which is seen a pink halo slightly raised and falling of from the centre towards the periphery to the level of the surrounding skin. The thickness is of about one millimetre.

Clinical Diagnosis. Trichophytia (?).

Mycological Diagnosis. *Microsporum lanosum.*

From these cases one may infer that locations on the surface of the naked skin are not uncommon. As to the origin of the disease, it is probably obtained from animals, chiefly from cats as in case no. 2, 3, 5 and 6. Contagion
from man to man is proved by case n. 4. Contagion from animal to man may also be seen in cases n. 2, 3, 5 and 6.

The third type, i.e. the mixed type, may be seen readily, by a glance at photographs n. 33, 34, 35. In n. 13 are seen microsporum-lesions of the scalp at the occiput and in n. 14 and 12 lesions of the skin surface are clearly seen.

Of this type I will report the following cases:

Case n. 7. (Photographs n. 33, 34 and 35.

A. A., 5 years old, brown, Rua January 1730, Brazilian. At school the disease is not reported. He became ill before the others. In April he noticed a pruritus on the skin. With this feeling a patch which became reddish put in its appearance. This lesion then appeared all over the body, the last one to appear being the one of the scalp. He says that the patches were at first red. Of late he had been treated with a preparation of camphor and alcohol, prescribed by a medical student. On the forehead the patient has a large patch over the right eye-brow and a smaller one over the left. On the face there are several others. On body, leg and arms, several others.

On the occiput, at the right side, there is a patch of about 2 cm. in diameter, with the hairs clipped short. Many pieces of hair-shaft show typical ringworm lesion. Production of small greyish scales. On the naked skin it is not easy to say that the hairs have been clipped, but a fine desquamation, as if follicular, is seen. The patch on the head has no odour. Broken hair are easily pulled out. From the periphery the hairs are long.

Clinical Diagnosis: Microsporia.

Mycological Diagnosis: M. audouinii.


About two months ago, or a little earlier, he noticed on his neck a very itchy patch, followed by other patches on face and scalp. The mother of the patient says that she already came ill from Ponte Nova, where he was born. She says that some time ago, at Ponte Nova, two of his brothers had the disease but readily overcame it. She says that on the ranch on which they lived lots of people had these patches. Pruritus intense. The little boy is very pale and frail-looking. On the face and neck are seen circular or oval patches, psudeochromatic on account of the dulling caused by the fine desquamation. On the head there are patches of alopecia about 2 cm. in diameter, circular, with the hairs broken off at the root.

The mother had only one miscarriage. On the patches of alopecia there are plenty of squamous and vesicular productions. The patches of the face and neck have in some parts visible scales. On these patches the hairs are still present. Material was taken for direct examination and cultures.

Clinical Diagnosis. Tinea tonsurans Ringworm of the scalp.

Mycological diagnosis: Microsporum lanosum.

Case n. 9. C. S., 5 years of age, white, Minas Geraes, Bello-Horizonte, pupil of the Kindergarten, was brought to the service on April 13, 1917, registered as n. 1681.

About 2 months ago he noticed several patches on the head which were treated by various remedies advised by druggists, without avail. No itching, pain or general symptoms have been elicited.

At present a patch is seen of 3 cm. diameter on the left fronto-parietal region, with clear, salient edges, hairs broken of short and covered with the ty-
pical sheath, easily plucked out with pincers and covered with greyish-white scales. The patch has an inflammatory appearance, probably due to the treatments applied, with tendency to vesicle-formation. There is another tiny patch at the vertex and another still smaller one on the occiput. There is yet another patch on the naked part of the nape. Over the cheek-bones, there are suspicious looking scaly-patches: (cf. phot. n. 10 of the lesion of the scalp).

Clinical Diagnosis: Tinea tonsurans.
Mycological Diagnosis: Microsporum Audouini.

These are the most clear cases of the mixed type which I wished to reproduce. Cases 7 and 9 were due to Microsporum Audouini as was proved by the cultures obtained, by examination in hanging-drop preparations and by the examination of hair-shafts and scales.

As is to be seen from the cases examined up to now, the lesions caused by microspora are at time pruriginous, at times not.

We will next reproduce some case-reports of type n. 1, that is of lesions exclusively located on the scalp.

Case n. 10. J., 5 1/2 years of age, Minas Geraes, Bello Horizonte, School for infants. Brought to this service on June 13, 1917. Reg. n. 1682.

More than 2 months and 15 days ago a patch was noticed on the vertex, without any pruritus or any special coloration. He was treated by a druggist without success. At present there is at the vertex a scaly patch, 3, 5 by 3 cms. in diameter, hairs clipped close. It is very little elevated. About ten patches of all sizes, spread over the scalp, often ill defined.

Clinical Diagnosis. «Tinea microsporica» (Ringworm of scalp caused by microsporum).
Mycological Diagnosis. Microsporum Audouini.

Case n. 11. R. J., 5 years of age, white, Minas Geraes, Bello Horizonte, rua dos Cabelés (in shacks existing in this street) n. 1672.

Three days ago her grandmother (her mother is in the ward with Pemphigus foliaceous) noticed a crown on the head of the patient, but was unable to say when it had first appeared. She states, however, that this must have happened within the last month. The little girl is unable to say whether it itches or not. It easy to infer that, if there is any pruritus, it must not be very intense. She has has had no other diseases. One of her brothers died from burns. Patch rigorously circular, about 2 cm. in diameter; it shows almost over its whole surface the hairs clipped off at from 1 cm. to 5 mm. from the surface. Many fragments of hair shaft are hidden below the scales, which are plentiful over the surface of the tonsured patch. Scales are greyish. This patch is on the vertex. There is a tiny patch hidden away under the hairs of the occiput. This patient has a little brother registered as n. 1671 in this service, for the same disease with the same location (case which I think unnecessary to reproduce).

Clinical Diagnosis. Tinea tonsurans.
Mycological Diagnosis. M. Audouini.
Case n. 12. years of age, brown, Minas Geraes, Bello-Horizonte, Rua dos Olotoni. Nov. 12, 1917. N. 1943.

She has had an affection of the scalp for about a month. At present many patches of uncertain form. Patches of alopecia are seen, in which the close-clipped hairs are not visible. When plucked out the hairs show the typical sheath.

Clinical Diagnosis, Microsporum Disease.
Mycological Diagnosis. M. lanosum.
Case n. 13. O. M., 8 years of age, brown, Minas Geraes, Bello Horizonte,
residing at Rua do Ramal. Does not attend school.

Came to the service on April 26th, 1917, reg. n. 1692.

There is already some time since there appeared on his head a white pruriginous patch, in which the hairs began to fall out. A patch is to be seen in the right temporal region. In it the hairs close-clipped. It has no special odour. The patch is covered with grey scales, easily lifted off. The patch is circular and of about 2 cm. in diameter. The patient still feels a great deal of pruritus.

Clinical Diagnosis. Microsporum Ringworm.

Mycological Diagnosis. M. lanosum.

This patient is a brother of the one registered in the skin and syphilis service as number 1942. His case is described in case report 14, which I will not reproduce here.

Case n. 15. M. M., brown, brazilian, residing in Bello Horizonte. Rua Pouso Alegre, school-boy.


About a month ago the patient noticed that the hairs began to fall off from a certain patch on his head. A few days ago he noticed that new foci were being formed. The skin in these places is thickened and covered with scales. There are a few hair-shafts in these clearly defined patches. Hairs close-clipped, with the characteristic sheath. There is no special odour.

Clinical Diagnosis. Microsporum Ringworm.

Mycological Diagnosis. M. lanosum.

Besides these cases, of which more or less: complete reports are at hand, I obtained cultures of M. lanosum and positive evidence by microscopical examination in cases n. 16 (J. H. M.) of which a photograph is given (Phot. n. 8), in which for the first time I noticed a peculiar odour, like the smell of rat’s nest, which is almost always felt in cases of patients suffering from Achorium schoenleinii; n. 17, J. L. M., brother of the former (n. 16) and of n. 18; n. 18 (C. R. M.) brother of the former. In these cases cultures of M. lanosum were obtained.

In the house of these three last patients there was a real family-epidemic. The reports of these cases are registered in the book of the Out-Patient Dep. for skin. Diseases and Syphilis (Prof. A. ALEIXO).

MICROSCOPICAL EXAMINATIONS OF HAIRS AND SCALES

Microscopical examinations should be made early, so that the best results be obtained. In more recent lesions, it may be asserted, it is always easy to show up the parasite. In older ones, however, repeated examination may be necessary. Even so, at times only a scant filament of mycelium is to be seen, and more often than not, after examinations repeated once, twice, three times, results are entirely negative. And thus one more negative result is applied to material from lesions which we know only too well to be caused by the fungus. As to methods, nothing more need be said. It is generally known and applied daily in this class of examinations. Once more let it be said that microscopical examination should be made shortly after the preparation is ready. The enclosing material (lus) used at present still leaves much to be desired, so that, if after a time the preparations are examined, the predominant appearance is that of a hair shaft from ringworm (there are plenty of air-bubbles). This is a probable source of error.

In a general way all hair-shafts examined show a great abundance of spores, at times ectothrix, at times endothrix. At times they are grouped in more or less isolated islets, in which about twenty to a hundred may be counted,
or, as happens not often, six to ten. Mycelian filaments are not always clear and easily seen, but in some hairs they show up very well and can be followed along a smaller or greater extension. At times they perforate the hair and come out. In these cases they can be examined in detail using principally Sahli’s blue which, without being first-rate, is one of the stains which must be made use of. With this process, at times metachromatic grains are to be seen within. Spores are, at times round, globose and oval, at times square, losengeshaped etc. These forms are brought about by the pressure brought to bear by spores lying near each other. They are also at times big, at times small. In some cases the giant-mycelia so well described by SABOURAUD are seen. In many instances the mycelia are not to be seen. At times the hair shaft is completely deformed at its extremities, in the middle, near the bulb etc. At the places in which the shaft is broken, real penicillate formations are seen in the hair. At times the hair-shaft, retaining more or less its structure, shows in certain places, as if it changed gradually or abruptly as to thickness. It is rare to see a completely regular shaft. In the scales from the scalp there is always to be seen clearly an infiltration by leucocytes.

In some hairs, the preparations of which were examined immediately after-mounting, I was able to see, like in the hair with favus, an enormous number of air-bubbles.

In the scales from the epidermal lesions, from the naked skin, it is not always possible, as has been already stated, to find the parasite. Only in fairly recent lesions is this possible. In cases n. 2 and 3 it was extremely easy. In these scales could be seen a broad, septeate mycelium, irregularly anastomotic, reminding one of the appearance of the trichophytes and also of Epidermophyton inguinale. I only came to a conclusion as to its determination after several inoculations on SABOURAUD’s culture media with maltose, glucose and crude molasses. The same thing happened in the case in which the air-bubbles were seen, like those seen in hairs with favus.

To sum up, microscopical examination is not sufficient by itself in the diagnosis of microspora; as often as possible culture-media must be inoculated so as to resolve the problem of their determination. Cultures in hanging-drop and direct examination of cultures are indispensable.

In the case of M. felineum the animal origin (cat) of the parasite was made clear.

There is one more fact that should remain of this survey of cases of Microsporum disease.

Not one of the patients was above 12 years of age.

ACHORION SCHONLEINII

I had the opportunity of studying four cases of Favus since February 1917.

I reproduce the following case-reports which are of some interest.

Case 1. M. A., ten years of age, brown, brazilian, residing in Bello-Horizonte, vaccinated, registered in this service as n. 18, 1918, attends school.

The mother lived with a syphilitic subject. She is undergoing treatment by mercury. Four years ago there appeared on the patients head an eruption of a yellow colour resembling says the patient ‘honey’. There was nothing on the skin. The patient had not submitted himself to any serious treatment. The disease spread until at some day he had lost all his hair. He did not transmit the disease, to his four brothers, nor at school. At present there is an extensive zone of alopecia occupying the

(1) See Photographs 26 and 27. Pleomorphic forms of M. lanosum and M. audouini.
upper part of the head (between parietal regions and vertex), leaving here and there tufts of hairs some of which are glued to crusts, which have replaced the pustules. Pustules are plentiful and contain a more or less thick pus. Some are clearly peri-follicular. On the right side the patch descends on the temporal region, only scales being seen together with the same appearance of alopecia. Another similar patch is found below and behind the vertex and another above the right ear. Tiny patches are spread about. The hairs are not easily pulled out.

On May 22nd., upon careful examination of some of the more recent patches, I saw that they were made up of a number of pale discs which the hair-shafts pierced. These were not found clipped. There is a very characteristic rat-smell. Hair-shafts pulled out bring sometimes the discs, in which a small depression is sometimes seen at the site of the follicle. Other larger discs can be seen with goblet-like depressions. On June 4th., 1918, a biopsy was made from the fronto-occipital region. (Cf. fig. phot. 38 for a better idea of what is herebrie- fly sketched out).

Case n. 2. W. S., 11 years of age, white, Brazilian, residing in Terras Novas. Was admitted to the service on Nov. 13, 1917. Parents dead. He has no other disease besides the one which brought him to the hospital.

About a year ago he noticed the appearance on the scalp of small swellings which suppurated soon and gave rise to a crust. He did not feel much pruritus in these swellings. The patient says that at home he was in contact with a horse but did not see any similar swellings on the horse. At present he shows on the upper part of the head great dry crusty patches, with obvious loss of hair. The latter is a type of alopecia which reminds one of syphilitic alopecia. The same lesions are seen further back and to a lesser degree at the sides. When the ochre crust has been lifted off a shining pink surface is seen. The crusts are fairly thick, some of them as much as two or three milli-metres. From the lesions there is exhaled a very characteristic smell of rat's nest. On the other parts of the body are seen small pink spots possibly due to mosquito-bites. A small desquamative lesion is seen on the two eye-brows. Nothing more is seen in the patient, who does not suffer even of the slightest degree of pyrexia. (For further details see photographs 41 and 40).

Case-reports 3 and 4 we will not reproduce. Of case n. 3, a patient of the female sex, photograph n. 39 is reproduced which will give an idea of her lesions.

CULTURES

In all four cases that we had the opportunity of describing, characteristic cultures of Achorion Schönleinii were obtained on Sabouraud culture-media with glucos, malt-sugar and with molasses.

These cultures were also imbedded and cut. Sections were stained with GIEOMSA, with haematoxylin-eosin, with toluidin blue, with SAHLI, as well as with the HEIDENHAIN and WEIGERT iron-haematoxylins, which are first rate stains and of great value for the identification (determination) of the parasite. Cultural examinations were also made by the dry-slide process, a technic attempted for the first time (A. NEVES). With the latter the morphology of the fungus was seen «au grand com- plet». Colonies on these slides are stel- late and very much akin to those of Sporotrichum Beurmanni (Photograph n. 53).

In them one may see intercalate chlamydospores of different sizes, at times pedunculated, false spores (pseudo-spores) in chains; the amoeboid forms described by SABOURAUD; the chandelier forms of favus; the pectinate organs etc. With this end in view, five or ten days after incubations of culture-media, after immediate fixation of the slides in sublimate-alcohol during 1, 2, 3 to 12 hours, we washed them in alcohol at 70° with iodine and them in alcohol at 70° pure, and we pla-
ced them in water until the alcohol had entirely disappeared. They were then ready to be stained for from half to one hour in GIEMSMA, and were next examined with an immersion-lens. Rod-shaped forms (bacillary forms) were not so often seen after this treatment. Beautiful chlamydomospores are seen and the preparations thus obtained are excellent for a comprehensive study of the fungus.

Of the cultures some photographs are given. N. 42 shows the original culture from case 1 (M. A.), after twenty days' growth. N. 43 shows the same culture from the side so as to show the elevation. N. 44 shows the culture in life-size corresponding to the same case of report n. 1 (all of them on malt-sugar SABOURAUD). Photographs n. 44 and 45 show cultures from case n. 2. In all four cases cultures were obtained. Photograph 47 is from M. A., 2 months' growth.

DIRECT MICROSCOPICAL EXAMINATION.

Microscopical examination was made of hair-shafts from the goblet-shaped depressions, of pus taken from the latter and of sections. In all of these the parasite was seen. We have stained preparations in which the parasite is easily seen, not only in the sections but also on the hair-shafts and in the smears of pus. In the section with the typical disposition; in the others with the beautiful structure and dispositions more or less modified by the crushing.

ACHORION GALLINAE (1)

It is already a long time that we have tried to find Achorion gallinae (MEGUIN et SABRAZES 1890 1893) in Bello-Horizonte. It would seem to be rare here. (2)

We found it in the fowl shown in the photograph shown (Fig 1).

We will not go into the historic part, botanical systematics etc. of this parasite.

We will just report what was seen.

The disease, in our case, was at first located almost exclusively, on the crest, with minute and few lesions elsewhere (breast and neck).

Later it developed on the body and legs.

The crest did not show any white spots and we feel obliged to point out this fact.

The colour was clearly yellow, like old gold.

Later by experimental inoculation, we were able to see that this is the colour right from the outset of the lesions.

Scales, bulbs of feathers, crusts had manifestly this colour.

The disease would thus be rather a yellow crest disease than white Crest of fowls.

SABOURAUD (Les Teignes, 1910, p. 557) uses the following words in describing the colour of the lesions:

«La tache blanche peut ainsi recouvrir la crête entière et sur ces deux faces, plus souvent la crête garde des parties saines. Plus une tache est âgée, mieux elle se détache sur le fond rouge de la crête».

The old-gold yellow of our case became more marked as it aged. When some of the affected feathers were plucked out, at the base of each, at the bulbar region, a real circle of definite colour, old-gold yellow was to be seen.

The disease had lasted some months, ans isolation, without any treat-

(1) Communication made to the Meeting of the Dermatology Clinic, June 30, 1920.

(2) After this we have been enabled to see other cases here.
ment, instead of improving the disease, made it worse.

Looking carefully at the lesions one had the impression of floury yellow crusts, covering the crest.

Scraping the lesions confirmed this impression. No goblet-shaped depressions (e.g., gobet's) were to be seen macroscopically.

Microscopic examination of the product obtained by scraping (prepared with lacto-phenol and potash) showed the presence of a lot of spores and mycelia of varying appearance.

Cultures on SABOURAUD's maltose culture-medium proved to be hard to obtain at first.

Association with ordinary fungi from crest and feathers explain this difficulty.

When the cultures of the real parasite begin to develop, already the medium has been taken hold of by *Mucor, Aspergillus* and so forth.

The technical artifice of previous washing out and shaking in sterilised physiological saline solution before inoculation of media obviated the obstacle, which is so frequent in such cases, of contamination by other non-pathogenic fungi.

Without displacing these others, it is impossible to obtain growths of *Achorion gallinace*.

Pure cultures of this fungus were snow-white, with the appearance of belonging obviously to the *Trichophyton* (Fig. 8).

This appearance was long retained. Age modified it, except as to colour.

Growth on dry slides (on a film of maltose-Sabouraud) furnished us with growths having the characters described by SABOURAUD (op. cit., page 568) for *Achorion gallinace*.

Cultures kept in the incubator at 30°C. for a number of days remained white.

Cultures in hanging drop (up to 41 days) showed a rich mycelial network.

We saw long septate mycelia with spindles in profusion, multiloculate and at first, without visible nucleus.

With prolonged observation we saw the nuclei appear in the spindles (70 days' growth).

Spindles had 5 or 6 loci.

There are minute external spores.

We inoculated, by scarification, these cultures on three men.

These inoculations gave no results.

Two fowls inoculated with the same material did not show any symptoms.

A fowl inoculated directly with the product obtained by scraping the crest of a sick fowl, showed, after ten days, typical lesions.

The lesion started with the formation of yellow crusts, fine and easily removed, and leaving after removal a damp red surface.

Other fowls, lying in the same chicken-yard, separated by a fine metal network, became ill as soon as the sick animal was introduced.

The sick cock died after a good many months, with a generalised dermatomycosis.

Almost all its feathers had a yellow circle round the bulb. The fowl was emaciated to a degree. Post-mortem examination did not show anything of interest.

Pathological investigations of the skin of the diseased fowl showed an appearance very similar to that described by SABOURAUD (op. cit., page 561).

In a depression of the stratum Malpighii there was a clump of parasites with the typical elements.

In the centre they made up a fine web, which thickened out into some of the peripheral branches.

We also saw, hardly broken up, the desquamating, horny layer, covering part of the lesion. Only once did
we find the typical appearance of goblet-shaped depressions ("godets").

In the remaining section, the parasites in regular masses, rested on the desquamated layer and were intermingled with it. We had the impression that in certain areas the parasite had dissociated the layers of the stratum disjunctum.

ALEUROPHORA BENIGNA n. sp. (1)

In 1916 one of us described a new parasite, related to Malassezia and, like this fungus, producing a benign skin-disease, hence Aleurophora benigna.

In that publication the chief characters of the lesions and of the parasite are described.

We may only add that its parasitism is not as extensive as was thought. It may be and must have been taken for Malassezia.

A type-photograph is given of the appearance of the cutaneous lesion (Photograph n. 54).

BLASTOSPOROUS FUNGI

The cases of blastomycoses were six in number. In the pus of the abscesses, in sections, in the beginning of some cultural growths the classical forms of blastosporous fungi were seen.

At times yeast-forms, round with typical double edges, at times typical budding forms with large or small buds. In the sections were seen the forms GASPAR VIANNA described and which have been so much described by North-American writers.

In smears of pus, we saw minute forms of round yeast-cells phagocyted by polymorphonuclears.

The appearance of the parasites in lesions reminded one of that described by HYDE and MONTGOMERY. The study of this group once more proved to us the necessity of a complete botanical study of the fungus (as well as the study of the clinical case) before a systematic determination of the fungus be made. It is enough to say that there have been cases, clinically classic types of the so-called blastomycoses, and in which the appearance of the cultures to the unaided eye reminded entirely of that of the blastomycetes, and in which, however, botanical study proved that there was no blastosporous fungus responsible.

One aspect alone is not enough.

It can show up, at most, one phase of the life of the fungus; 2d the unreasonableness of the present division of the group of the Blastomycoses.

Already VUILLEMIN and DE BEURMANN and GOUGEROT went in to this subject with greater criterium. The macroscopic appearance of the cultures, on the classic SABOURAUD medium, was powdery, wrinkled, and therefore aburring from BUSSE's type in 4 cases. In the remainder the appearance was that of yeast (the classic appearance).

The complete botanic study of these parasites may perhaps be the subject of a future paper by one of us (A. NEVES).

CONCLUSIONS

I

General Conclusions:

a) Macroscopic Appearance of the fungus-cultures:

1. The general macroscopic appearance of a fungus-culture, on the culture-media generally used, is not decisive for the diagnosis of a species. Often enough this appearance is precarious.

2. Pigmentation of fungus-cultures on ordinary laboratory-media, cannot be taken as a decisive element in the determination of pathogenic fungi.
3. As good technical methods for species diagnosis, the «dry slides», combined with SCHROETTER’S technic and the classic SABOURAUD media, is what appears to give best results.

4. Smear preparations, as a means of studying the morphology of fungi, are always faulty. They are useful, however, in helping one out.

5. The combination of all the processes which can be used in the laboratory, is what, in the present state of our knowledge, can give the greatest number of positive results in mycological diagnosis.

b) Microscopic Appearance of cultures:

6. In this field good results will come from the greater number of methods used.

7. Often enough on one «dry slide», the whole botanical picture of the fungus will be found.

8. Rod-shaped (bacillary) forms, far from being anomalous, are quite common in cultures of pathogenic fungi (as in lesions caused by these fungi).

9. The process of fixation without drying in sublimated alcohol and staining with GIEMSA gives first-rate results.

c) Human Material for Examination:

10. Material taken from Man must always be obtained with the greatest aseptic precautions.

11. Microscopical examination of material taken from lesions caused by fungi must be oft-repeated to obtain uniformly good results. Three examinations are not always sufficient.

d) Biological Reactions.

12. Biologic Reactions should, when possible, be tried as a subsidiary means, which may be of great value in obtaining the final result of the investigation.

SPECIAL CONCLUSIONS:

II

c) Microspora.

13. Clinically it is not possible to make a differential diagnosis between the different known species of microsporum.

14. On the classic SABOURAUD culture-medium, the external appearance of Microspora, is a great help in the determination of species.

15. On other culture-media the appearance is varying; they must not however be cast aside.

Microscopical examination of the cultures is of great value in the determination of the species and of the origin.

17. The microscopical appearance of material from the lesion is varying; it is generally of no use in the making of an exact diagnosis.

18. Microspora conditions here (in Bello Horizonte) appear to be limited to children.

f) Sporotricha.

19. In thirty-one cases in which we obtained growths of Sporotrichum, the fungus belonged in every case to one species, Sporotrichum Beurmannii.

20. Only in three out of the thirty-one cases studied were parasites more or less plentiful in direct examination of the material.

21. The surest method of diagnosis is by inoculation of culture-media.

22. Rod-shaped and coccoid forms are frequent in tissues infected by sporotricha.

23. Biologic tests, complement-fixation, sporo-agglutination, do not always agree with the positive results of the cultures.

24. Of the biologic tests, the second should be preferred, since it gives the greater number of positive results.
g) ALEUROPHORA n. g., n. sp.

25. The disease caused by Aleurophora benigna is allied to Pityriasis. The fungus, however, is aleurosporate.

h) Malassezia.

26. Clinical symptoms of this disease are not pathognomonic.

27. Microscopical appearance of the parasite in the lesions may vary. It easily looked for.

28. Negative cultures in the case of Malassezia are good evidence in favour of the diagnosis.

i) Trichophyta.

29. In Bello-Horizonte trichophyta are very much rarer than microspora (one of the former to 19 of the latter).

j) Favus.

30. Air-bubbles in microscopic examinations and in direct examination of the hair-shafts are not pathognomonic of Achoria.

31. Cultures of the four cases of our statistics were easily obtained. Pleomorphic forms were never seen.

k) Microsiphonata.

32. Mycoses produced by microsiphonata are rare in Bello-Horizonte.

l) Oidiosis.

33. Lesions, especially in the lungs, caused by Oidium braziliense are not very rare.

34. This fungus produces, in man as in animals, lesions very similar to those of tuberculosis.

35. Treatment with iodids is specific against this oidiosis.

36. There is in the cultures of Oidium braziliense a form of vegetative cyst similar to that found in Coccidium immitis. The results of division are however rod-shaped elements which begin anew the life-cycle of the parasite.

To Prof. EZEQUIEL DIAS our most sincere thanks for the facilities made for us in the Branch of the Instituto OSWALDO CRUZ in Bello-Horizonte.

To Prof. MARQUES LISBOA and to Dr. OCTAVIO MARQUES LISBOA our indebtedness for their kind help in the making of microphotographs.
LIST OF PHOTOGRAPHS AND DRAWING.

Phot. n. 1. From case n. 1. Case of Sporotrichosis. Primary lesion (the sporotrichotic sore is not visible). Lymphangitic and cutaneous lesions conspicuous.

Phot. n. 1-a. Same case. Primary sporotrichotic sore.

Phot. n. 2. Case of J. M. Sporotrichotic lymphangitis.

Phot. n. 2-a. Culture from the case of J. M. Three months and 17 days of development. Classic type of Sporotrichum-culture.

Phot. n. 2-b. Primary sporotrichotic sore. Case of J. M.

Phot. n. 2-c. Another view of the lesions of J. M.

Phot. n. 3. Growth on maltose-Sabouraud from case n. 1. Classic appearance, 47 days' development.

Phot. n. 4. Case of X. Culture of Sporotrichum. Classic type. 14 days of development.

Phot. n. 5. Case of A. L. Culture of the classic type, with 60 days of development. 1912. This is believed to be the first case of cultures obtained in Bello Horizonte.

Phot. n. 6. Growths without furrows, white and powdery of Sporotrichum.

Phot. n. 7. C. M. Sporotrichotic sore and lymphangitic lesions. Spontaneous healing.


Phot. n. 9. Another view of the same lesions. Patient n. 4536.

Phot. n. 10. Sporotrichosis. Polyclinic of Prof. Dr. ALEIXO. Out-patient 5081.


Phot. n. 16. Pleomorphic culture of E. inguinale (from scales found in arm-pits).


Phot. n. 27. Microsporum lanosum. B. J. Epidermal scales. 14 days of development.


Phot. n. 36. Culture of *Microsporum Audouini* from hairs. This belongs to Out-Patient 1730.


Phot. n. 42. Culture from a hair-shaft of M. A. on glucose-Sabouraud. Lateral view slightly magnified.

Phot. n. 43. Culture of *Achorium Schoenleinii*. Twenty days of development. Test culture-medium.

Phot. n. 44. Culture, case of patient 5511.

Phot. n. 45. Culture, case of patient 5511.


Phot. n. 47. Growth on dry slide of *Oidium brasiliense*. Ocular 5, obj. AA.

Phot. n. 48. Growth on dry slide of *Endomyces albicans*. Oc. 5, obj. AA.


Phot. n. 52. Case of G. III for 15 years. *Neurophora benigna*.

Phot. n. 53. Cock with yellow crest.

Phot. n. 54 and 55. Quills with yellow necklace produced by *Achorium gallinace*.

Phot. n. 56. Ten days growth of *Achorium gallinace*.

Phot. n. 57. Twenty days growth of *Achorium gallinace*.

Phot. n. 58. Section of the skin of the cock shown above, typical goblet-appearance.

Drawing n. 1. Beginning culture on maltose-Sabouraud of *Oidium brasiliense* transplanted from the dry-slides.