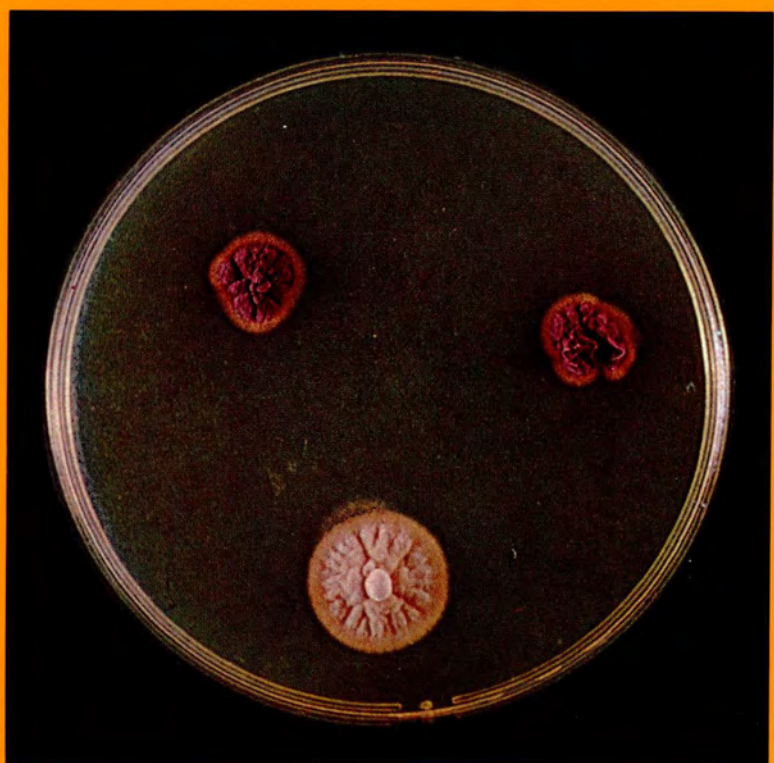
Laboratory Mycology

H. Rieth



BIOTEST

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Foreword

This booklet was instigated by the many encouraging suggestions from dermatologists who work with ready to use BIOTEST culture media for the cultivation and diagnosis of dermatophytes, yeasts and moulds. This publication therefore proves that the many letters received and the stimulating conversations held at exhibitions have had the desired effect.

When we told Professor Rieth of our customers' wishes for an introduction into mycology we found support and co-operation. We are grateful to Professor Rieth, not only for his trouble in taking on a publication such as this, but also for the enthusiasm with which he approached the work and which he instilled in us at the same time.

With the introduction to "Laboratory Mycology" our aim is to take the first step in gaining for medical mycology the place it deserves within the wider field of microbiology.

BIOTEST-Serum-Institut
Department of Microbiology

Contents

ILLUSTRATIONS

I. Obtaining and preliminary treatment of material

A. Skin and cutaneous appendages

1. Skin scales

Before obtaining skin scales the lesion is thoroughly cleansed of surface deposits. For this, absorbent gauze is more suitable than cotton wool or cellulose. If no large defects in the epithelium or fissures in the skin are present, light sponging with 70 % ethylalcohol or isopropylalcohol is recommended. In this way false positive results due to airborne organisms are avoided, and bacterial growth is considerably reduced.

The large scales should be removed first of all and discarded; they are of no interest. The small scales on the edges of the lesions are taken off carefully with a scalpel or surgical spoon in the direction of the healthy skin.

Altogether at least 30—40 smallish scales should be transferred into a sterile Petri dish or a sterile, widenecked tube. In difficult environmental conditions sterile bags suitable for transportation can also be used.

Before the scales are inoculated onto the culture medium they must be cut into very small particles, and this is done best with a scalpel and dissecting needle. The instruments must be heat-sterilized beforehand, i. e. in a glass flask for 2 hours at 180° C. Care should be taken in preparing a sufficient number of sterile instruments.

Warning: The popular method of drawing scalpels or surgical spoons through a flame leads either to quick deterioration of valuable instruments (as they have to be completely heated through), or to incomplete sterilization with the risk of contaminating samples with fungal elements from previous patients. The false data which then arise can only be avoided by correct sterilization.

2. Hairs

The hair-covered lesions which can be caused by fungi, e. g. on the head, face, back of neck, trunk and limbs, must also be cleaned carefully with 70 % ethylalcohol or isopropylalcohol. All crusts and scales around them are removed, preferably with a scalpel or surgical spoon. Those hair-stumps which are to be removed with epilating forceps must then be found, if necessary with a magnifying glass. This can be carried out at the edge of a bald-patch or even on the lesion itself if single suspected hairs are to be found there, e. g. in the case of trichophytosis, or so-called "mown-meadow", which is typical for classic microsporosis. In this instance practically all the hairs of the focus are broken slightly above the level of the skin.

Altogether at least 20, preferably 30—40 hair-stumps are needed per case.

Important:

Cutting hairs with scissors and having these mycologically examined is of no use at all and is only proof of ignorance.

Contaminated hairs

If the hairs are extremely soiled, e. g. with pus in the case of Celsus' kerion or mixed infections, washing the short hair-stumps in a sterile fluid is recommended before inoculation. For this purpose antibacterial antibiotics such as penicillin, chloramphenicol, etc. can be used in the concentrations employed also in corresponding culture media.

Animal hairs

Animal hairs are very often contaminated with additional mould fungus spores. The hairs which are most frequently examined in the mycology laboratory are from cats, dogs, guinea-pigs, rabbits, hamsters, horses and cattle. It is almost impossible to carry out the cleaning process with 70 % alcohol on animals. Furthermore, plucking out single hairs with epilating forceps is difficult and only possible in certain cases. It has proved satisfactory to brush infected areas with a sterile toothbrush, remove the hairs which remain hanging in it, and then dip the toothbrush straight into the culture medium. Cycloheximide should be added to this medium in order to suppress too rich growth of mould fungi.

3. Nails

With suspected fungus infection of the nails, it must first be determined whether the fungus has attacked the nail-plate and hyponychium, or whether it is a case of mycotic paronychia with possible secondary lesion of the nail-plate.

Fungus infection of the nail-plate

If the suspected mycotic area is in or underneath the nail-plate itself, all visible infected tissue must be removed with a sharp sterile scalpel and the junction between healthy and infected tissue must be exposed. Then the cleaning process must be carried out with 70 % alcohol and a gauze pad.
Very fine nail particles are taken off with a scalpel or surgical spoon. A fair amount of material must be obtained here too as the fungi are hidden in nidal form and cannot be detected with the naked eye.

Warning: Cutting nails with scissors and then sending a piece to the laboratory is of no use whatsoever, and should be prevented by providing information for those concerned in sampling.

Paronychia

With suspected mycotic paronychia it is recommended that material from the suspected areas, which is almost always purulent, be taken carefully with a wire loop or mycological hook (an unbent wire loop), and placed immediately onto a solid medium or into a fluid medium. It is helpful to moisten the wire loop with the medium first of all, so that the fungal elements stick to it. Material for direct microscopic examination is placed straight onto a slide. It is necessary to break down the nail material with a scalpel and dissecting needle into smaller pieces if the particles are larger than about 1 mm. This is most easily achieved in a sterile Petri dish of which the lid is opened just a little on one side.

Extracted nails

Extracted nails sent to the laboratory are very rarely free from contamination. It is best to clean the nails carefully (e. g. with penicillin or chloramphenicol solution) in order to eliminate any bacteria which may have stuck to them. The areas suspected of fungus attack must then be carefully sought. Material for the culture should only be taken from several different such places.

Important: Extracted nails must never come into contact with formalin.

B. Urogenital tract

1. Vaginal secretion

Obtaining can be carried out in different ways: with a wire loop, a swab, a surgical spoon or a speculum. Preliminary treatment of secretion is not necessary; nevertheless there is no harm in taking precautionary measures against dehydration of the material during transport, e. g. by using swabs soaked in nutrient medium or by placing the secretion in sterile physiological saline solution.

2. Swab from the vulva

According to the form of disease proceed as described under "Skin", or take as much material as possible using a wire loop or smear swab.

3. Contact culture from the penis

With suspected mycotic balanitis or balanoposthitis bring the glans penis or the prepuce into direct contact with the surface of the medium in a Petri dish. Press carefully several times so that the surface of the medium is inoculated in different places.

4. Urine sediment

Urine removed under sterile conditions (or mid-stream urine) is centrifuged in sterile tubes, then poured out quickly so that only a few drops remain at the bottom of the tube. These drops are either used directly or by means of a wire loop.

It is important to note that when sending urine into the laboratory, no sort of disinfectant, especially formalin, should come into contact with the urine or the container used for transport.

5. Urinary tract secretion

Smears from the orificium urethrae or from the urethra are taken with a wire loop or cotton applicator.

C. Digestive tract

1. Oesophageal swab

Material is obtained by oesophagoscopy from suspected areas and examined immediately or placed into 1–2 ml physiological saline solution.

2. Gastric juice

Material obtained by means of a gastroscope or pump is taken into a sterile container, if necessary diluted with a little physiological saline solution, and centrifuged in a sterile tube. Only the sediment is then processed.

3. Duodenal juice and bile

Proceed as under"Gastric juice".

4. Faeces

A hazelnut-sized part of a stool portion is brought for mycological examination, after the stool portion has been mixed in 20–30 places with a spoon fixed to a stopper.

D. Mouth and pharynx

1. Swab from the oral cavity

Smear as large as possible an area of the oral cavity or the suspected mycotic areas with a cotton applicator, if necessary with the help of a wire loop. Gum pockets and the tongue, especially the edge of the tongue, must not be overlooked.

2. Dentures

Contact-cultures are best. Here the plate is pressed directly onto the surface of the culture medium, if possible in several different places.

3. Swab from the pharynx

Coat the cotton applicator thoroughly with pharyngeal secretion. Always smear several different places more than once.

4. Matter expressed from the tonsils

The contents of the crypts are carefully expressed and taken into sterile physiological saline solution. Material can also be obtained by suction.

5. Saliva

Pathogenic yeasts do not belong to the normal mouth flora, but the possibility of colonisation of the oral cavity can make the evaluation of results of the sputum examination more difficult. Examination of the saliva is necessary as a control. Saliva can be diluted, according to its consistency, with sterile physiological saline solution.

E. Respiratory tract

1. Sputum

When obtaining sputum it is very important not to mix it with saliva, or to minimise admixture. To do this it is best to make the oral cavity as free of fungi as possible for one or two days before obtaining material, e. g. by sucking throat-pastilles or by thorough mouth-washing with appropriate solutions.

Sputum is coughed into a sterile wide-necked vessel or a sterile Petri dish. Dilution with sterile physiological saline solution can be useful if the sputum is too viscous.

2. Bronchial secretion

The secretion obtained with a bronchoscope is taken into a sterile tube with the help of a sterile rinsing liquid and is submitted for examination without formalin, penicillin, etc.

3. Fluid from the pleural cavity

This is put into a sterile tube without additional substances, e. g. formalin, penicillin, etc.

F. External auditory canal

1. Smear

Obtain material with a cotton applicator or surgical spoon and place it straight onto the culture medium or in a sterile vessel.

2. Cerumen

Remove with a surgical spoon, pincet or wire loop and place it in a sterile container. Work carefully if moulds are seen to be appearing in order to avoid the spreading of fungus-spores.

G. Spinal fluid

Place without added material in a sterile container and centrifuge in a sterile tube. Inoculate sediment only.

H. Blood

5—10 ml of venous or arterial blood is taken into a sterile container and sent to the mycological laboratory without any other added material. It is best to inoculate 40—50 drops straight onto the solid medium about ½ cm away from each other. The "Micrognost Blood Culture Bottle" facilitates withdrawal and transport.

I. Biopsy and autopsy material

It is extremely important to state that material to be examined must never be placed in any bactericidal fluid, especially formalin, before being sent to the laboratory. Such treatment would preclude the growth of fungi.

However, in order to prevent drying out the sample may be placed in sterile physiological saline solution. To control or stop the spreading of any bacteria, antibacterial antibiotics can be added, e. g. chloramphenicol in a concentration of 50—200 mcg/ml.

II. Inoculation of the specimens on various culture media

A. Solid media

1. Kimmig Agar

A large amount (20–30) of finely cut skin scales, nails or hairs are distributed on the medium in such a way that the single particles are at least a few mm away from each other. The purpose of this is to prevent a too rapid growth of contaminants over the whole area of the inoculated material. Kimmig Agar leads to very good growth in the case of most pathogenic fungi with rich formation of microconidia and macroconidia without encouraging pleomorphism (development of cotton-type sterile mycelia). Kimmig Agar is therefore well suited for all primary cultures which are not particularly contaminated.

2. Selective Agar for pathogenic fungi

Inoculation is carried out as with Kimmig Agar. The purpose of adding cycloheximide is to restrict certain mould fungi so that they do not grow too quickly over, or even completely suppress, the slow-growing dermatophytes. However, it must be taken into account that mould fungi may also be the causative pathogens.

Furthermore it is important to note that some pathogenic yeasts are likewise suppressed by cycloheximide, leading in some cases to false negative results.

With samples suspected of contamination, Selective Agar containing cycloheximide is highly recommended. In order not to overlook pathogenic moulds and yeasts which are sensitive to cycloheximide, parallel cultures must be made on media not containing this antibiotic.

It is best to inoculate two solid media in each case (not one solid and one fluid) — one with cycloheximide and one without.

3. Sabouraud Dextrose Agar

A traditional medium containing double the amount of sugar as Kimmig Agar. Therefore on the one hand pleomorphism is encouraged where it is not desired, but on the other hand the formation of pigment is in many cases influenced advantageously.

Sabouraud Dextrose Agar is especially suitable for subcultures if clear identification is difficult with the primary culture. In many cases the growth forms on two different media allow a better determination of the range of growth forms.

4. Candida Agar

A relatively quick result can be obtained using this medium in the case of suspected pathogenic yeasts. The growth within a few days of small, dark, almost black colonies, which consist of yeast cells when observed under the microscope, proves that yeasts were in the specimen.

The material is spread well over the surface of the medium with a wire loop or other instrument.

Important: The original assumption that only Candida albicans would grow on Candida Agar has not proved to be true. Also yeasts growing on Candida Agar do not necessarily belong to the yeast genus Candida. In fact yeasts belonging to the genera Torulopsis, Trichosporon and even Saccharomyces also grow on Candida Agar. When this is taken into account, false mycological diagnoses are prevented.

The main advantage of Candida Agar is that a characteristic growth is quickly obtained from which yeast infection can be determined. With urine sediment or vaginal secretion, where almost all detected yeasts are pathological, it is therapeutically time-saving even if the genus and species are not determined.

5. Rice Agar

If it is necessary to identify the yeasts, diagnosis of Candida albicans must first be carried out. For this, various special culture media are suitable, especially the so-called Rice Agar, which needs careful production and is therefore better obtained as a ready to use medium..

Inoculation is easy. Just a little material from a previous culture, e. g. from the brown-black colonies on Candida Agar or from the whitish-grey or whitish-yellow colonies on Kimmig Agar, is inoculated in wide snake-like lines or in three streaks 1 cm apart from each other. Then one or two coverglasses are placed on each of the streaks. Under the glasses a semianaerobic growth is induced with rich pseudomycelia formation and development of chlamydospores typical for Candida albicans. The closely related Candida stellatoidea forms similar chlamydospores, but the other Candida species (at least 79) do not.

True mycelia as well as pseudomycelia grow on Rice Agar, arthospores also form, and ascospores of the perfect yeasts occur.

Important: Store Rice Agar cultures at room temperature only. At 37° C in the incubator the characteristic growth forms are greatly restricted or do not occur at all.

B. Fluid media

Sabouraud Medium

This medium is especially suited when enrichment of fungi, which are perhaps only sparsely distributed, is required.

Inoculation is carried out usually with a wire loop, but may also be done straight after obtaining the material with a scalpel, surgical spoon or cotton-applicator.

III. Subcultures

By subcultures one understands all those cultures originating from primary cultures by transfer of particles onto another solid or fluid medium.

Subcultures serve various purposes, one of which is to separate mixed cultures on the original plate. A second inoculation onto a differently constituted medium allows the determination of different growth forms; this can make identification of certain dermatophytes much easier.

It is also a good idea to make these so-called identification cultures in duplicate – by making a monoculture in the middle of the medium in one Petri dish and a triple culture in a second Petri dish. The reason for this is that the fungal colonies behave differently – both by strain and species – according to whether they are influenced by neighbouring colonies or not.

Subcultures in the form of pure cultures are also important for the strain collection of fungi (mycotheque). When there are as many pure cultures as possible available for comparison, identification is easier and the diagnostic result is thus safer. Furthermore, time is saved as contact with other mycological laboratories for further information is not required so often.

Subcultures also serve in determining sensitivity and in testing new substances for antifungal activity.

Subcultures can alter their appearance as a result of exogenic or endogenic influences.

IV. Incubation

A. Place of incubation

In general, incubation takes place protected from strong sunlight, air-turbulence and dust; cupboards which can be securely closed and are protected from light are better than shelves, tables, window-sills or racks, for cultures in Petri dishes; tube-cultures are less at risk from the environment. However, by re-sealing Petri dishes with the sealing tape (leaving a gap of 1—2 cm to allow air flow) the danger of pollution from air currents is reduced. With higher temperatures incubators are best.

B. Length of incubation

This is usually between 3 and 30 days. In special cases — if it is urgent and only yeasts are to be tested for — 24 hours can be enough for the incubation. Otherwise with suspected yeasts the recommended length of incubation is up to 8 days.

Moulds grow at different rates; some are well developed after 5—8 days, others take 3—4 weeks.

Dermatophytes usually grow within 1—3 weeks; sometimes however it can take a little longer, if for example the fungi have been injured by immunological reactions of the host or by medication.

With primary cultures, where identification of the growing fungus is not obvious, the cultures should be observed for 4 weeks, even if after a short time fungal growth has already been established. The reason for this is that it could be a multiple infection, or the first fungal growth could be coincidental contamination and the actual pathogen might not appear until later.

It is recommended to incubate fungus cultures a little too long rather than evaluate too early. Giving out preliminary results can meet the expectation of patients or doctors sending material into the laboratory without foregoing the possibility of completing these results or making them more precise after 3 or 4 weeks.

C. Temperature of incubation

In general, incubation takes place at room temperature – about 20°–25° C. In certain cases incubation at 37° C is important, so an incubator in the mycological laboratory is very useful.

Fungi which do not grow at 37° C cannot increase inside the body so this is an important criterion with suspected deep mycoses. However on skin and in its appendages where temperatures are much lower, those fungi flourish greatly which do not grow at all or only at a reduced rate at 37° C.

Important: It would be completely wrong to incubate only at 37° C on principle. If enough material is available then parallel incubation at room temperature and at 37° C in the incubator is the best solution.

Incubation at about 30° C.

Dermatophytes grow most quickly at 28°–30° C. Those who have an additional incubator for this temperature can gain a few days by quickening the growth of the material in primary cultures by incubating at about 30° C.

Incubation in the refrigerator.

There are some moulds which form toxins at refrigerator temperature, whereas at higher temperatures this is suppressed. Mycotoxins can be absorbed percutaneously and can have a hepato-toxic effect. It is not advisable to store mould cultures in the refrigerator as one cannot be sure whether toxin is formed or not.

V. Identification criteria

A. Morphological criteria

1. Habitus

"Habitus" means the physical appearance of a fungus culture, especially the surface structure. Many growth forms can occur, either caused by the different substrata or endogenously. Many fungi behave quite differently in their various growth forms so that identification is made more difficult. The following statement is to be noted in the laboratory:

the same fungi can look different;
different fungi can look the same.

For this reason it is necessary to get to know the range of growth forms of the most common fungi.

2. Colour

Pigment formation can depend on the aerial mycelium as well as on the substrate mycelium. The mycelium can be different in pigment from the sexually reproduced perfect forms or the asexual imperfect forms. With certain fungi the pigment can diffuse into the culture medium.

One must take into account that the formation of pigment can also be suppressed, for example with Trichophyton rubrum. It may be supposed that the name is determined by the red colour of the fungus, but this is not the case. Most strains of Trichophyton rubrum are downy-white and only on the underside is there a more or less distinct red-violet colouring. Many other fungi form red colouring without necessarily being Trichophyton rubrum.

3. Reproductory organs

a) Sexual reproduction

Some dermatophytes, yeasts and moulds form sexual organs and therefore belong to the perfect fungi which can be classified in a natural system.

In fungus cultures such fruit bodies can be observed sometimes, e. g. Perithecia (with pre-formed opening for freeing the spores formed inside the body) or Cleistothecia (closed on all sides; the spores are freed by the bursting of the body).

b) Asexual reproduction

Asexually reproduced forms are very common and are called imperfect forms (in contrast to the sexually reproduced perfect fruit body). The most well-known asexual "spores" are conidia. With dermatophytes and with a few moulds one differentiates between small microconidia and larger macroconidia which are usually multiseptate.

The conidia can come straight out of a fungus-thread or stand on small stalks; they can be smooth or rough, longitudinally or transversally septate, pointed or flat, club-shaped, spindle-shaped, cylindrical, bizarrely misshapen, round, oval, pear-shaped, banana-shaped, etc.; in view of the many possibilities drawings of the most common shapes are a great help in evaluating the different criteria.

If a fungus-thread breaks into limb-like pieces one talks of arthrospores; these "mycelium spores" also help in reproduction, although of course they are strictly speaking not "spores".

With yeasts, blastospores are especially important. The blastospore is the normal vegetation body of a yeast, in other words not a "spore" in the proper sense of the word. Yeast cells can reproduce by budding, the daughter cell buds out of the mother cell which it resembles as soon as it is completely developed. Blastospores are then only budding cells; they can be round, short-oval, long-oval or elongated, they can appear triangular, ogival or bottle-shaped; or they can adhere to each other, in which case they are called pseudomycelium. A deciding criterion is often the formation of chlamydospores.

c) Dimorphic fungi

A number of fungi can change their form. Under certain conditions, e. g. in the tissue and at 37° C in vitro, budding cells are formed; under other conditions, e. g. at room temperature, these fungi grow in threadlike form, partly as mould fungi.

One must always take into account when evaluating the identification criteria that a fungus can be both a "thread fungus" as well as a "budding fungus". Grouping into "thread-fungi" and "budding fungi" held true only for a short time in a simplified theoretical didactic – in practice it has not been proved satisfactory. In the laboratories it is becoming more and more common to make a distinction between dermatophytes, yeasts, moulds and other fungi. Both yeasts and moulds can be dimorphic. Even dermatophytes can reproduce by budding under certain conditions.

B. Physiological criteria

1. Fermentation

For the determination of yeasts, which one cannot identify by morphology, various metabolic reactions are suitable identification criteria, especially the fermentation of sugars.

Firstly 5 sugars are tested: dextrose, galactose, sucrose, maltose and lactose. If it is still not possible to identify the yeast and the assimilation tests also fail, then further sugars are tested.

Details about the technique of sugar fermentation may be obtained from specialized literature.

2. Assimilation

a) Assimilation of sugar

Here too 5 sugars are used first: dextrose, galactose, sucrose, maltose and lactose. In certain cases additional assimilation tests are carried out.

b) Assimilation of nitrogen

Peptone and potassium nitrate are tested according to standard methods. Peptone is assimilated by all yeasts, potassium nitrate only by those which can use an inorganic nitrogen source.

3. Further metabolic reactions

In certain cases the splitting of arbutin is tested. Also the utilization of urea, fat splitting and keratinophilia, etc. can be important.

C. Serological criteria

The routine use of serological criteria for the identification of fungi is still being discussed. Significant research results indicate that the identification procedure could be shortened in some cases by serology.

D. Fluorescence immunological criteria

In special laboratories excellent procedures have already been developed to detect certain fungi, e. g. Sporothrix schenckii, if other procedures fail. In a few years this method will be feasible in well-equipped microbiological laboratories having an equally well equipped mycological department.

VI. Fungus differentiation and classification

For the identification of a fungus the differentiation from similar looking related or non-related genera and species must be exact and reliable. Differentiation is facilitated in that the various fungus species (numbering over a hundred thousand) are classified into systems.

Since Linné fungi (like all plants) are classified according to sexual organs; this is the natural or perfect system. If the sexual organs are not known or not formed, the fungus strains are sorted into an artificial or imperfect system on the basis of other criteria, e. g. conidia formation. The artificial system is also known as the second system. The imperfect fungi are also called deuteromycetes after the Greek word "deuteros" meaning second.

For the distribution into dermatophytes, yeasts and moulds the distinction between "perfect" and "imperfect" is in practice of secondary importance.

The perfect and imperfect forms are named differently, for example Keratinomyces ajelloi (imperfect) and Arthroderma uncinatum (perfect) are not – as one would suppose – two different fungi, but one and the same organism. It does not matter which of the forms grows on the medium – one knows what the other form should be called. As a result the classification of dermatophytes is quite unproblematic as long as it is applied to either the perfect or the imperfect stages.

Similarly with yeasts and moulds most problems of this kind are solved in the laboratory without any very great difficulties. However, in research into the relationships between the various fungi the sexuality is very important.

A. Dermatophytes

1. Microsporum

Fungi of the genus Microsporum have rough-walled macroconidia. Identification on the basis of this criterion only produces difficulties as they often do not occur at all. It is also misleading if the macroconidia have lost their rough protuberances in the test fluid between the cover and the slide, because then of course they become smooth. This has led for decades to the incorrect identification of Microsporum ferrugineum as Trichophyton ferrugineum.

a) Microsporum audouinii

Formerly the most common pathogen of the microsporic endemics and epidemics. Today it is very rare in Europe, but in other countries, e. g. in West African states it is not yet eliminated. Diseases caused by this organism can easily be imported from abroad.

b) Microsporum canis

Common all over the world, this fungus occurs much more often in Europe than is generally supposed. Infection of man from various animals and vice versa can be avoided by careful hygiene measures.

c) Microsporum gypseum

A common pathogen of "Gardner's Microsporosis"; occurs in nidal form in the soil and is therefore a geophilic fungus.

It is important to note that this fungus does not cause the same clinical picture as M. audouinii or M. canis. Also hairs attacked by M. gypseum do not fluoresce with a yellowish-green colour as with "classic" microsporosis.

d) Other species of Microsporum

If required further details about these will be given in a later edition.

2. Trichophyton

Fungi of the genus Trichophyton have smooth-walled macroconidia (if any are formed at all). This is where the dilemma lies in determining Trichophyton: one very often seeks macroconidia which are not present. Therefore other criteria are necessary in such cases.

a) Trichophyton mentagrophytes

Formerly the most common cause of mycosis of the skin of the hands and feet, today it takes second place in many countries. It is assumed that Trichophyton mentagrophytes is identical to the so-called Kaufmann-Wolf-fungus of former times, or at least partly so.

Very common in animals, both in the wild and in captivity. Infection can result straight from the soil but can also of course be transferred through various animals, e.g. guinea-pigs which are very popular pets.

b) Trichophyton rubrum

In many countries the most common cause of dermatomycoses of smooth and lanugohaired skin. Also occurs in the hair of the head and beard. Probably identical with Kaufmann-Wolf-fungus in many cases, especially when white-downy cultures do not form any pigment.

c) Trichophyton verrucosum

Especially common in country areas as this fungus occurs predominantly in cattle. Children and calves are affected most. Towndwellers can be affected after holidaying on the farm. T. verrucosum grows extremely slowly. For this reason one should not make a final evaluation of cultures in less than 14 days. One can even wait three weeks for this fungus to develop.

d) Trichophyton violaceum

An especially variable fungus. The culture growing out of scalp hairs are most typically violet. Very common in the Middle East and Africa it occurs in practically all the Mediterranean countries. Endemic in Europe. Children and adults alike can be affected.

It is sometime difficult to identify the pink or downy-white variants so every laboratory should be supplied with several cultures for comparison.

e) Other Trichophyton species

If required the rarer species will also be described and illustrated in a later edition.

3. Epidermophyton

In the genus Epidermophyton which is distinguished by smooth but typically club-shaped macroconidia (at the same time as complete absence of microconidia) there exists at the moment only one single species.

a) Epidermophyton floccosum

Characteristically the culture is greenish-grey with yellowish and even reddish tinges here and there, and after growth of about 1–2 weeks small white flocks of cottonwool-type aerial mycelia develop.

It mainly occurs in the genitocrural area and groin, but can also occur on the limbs, on the trunk und even sometimes in the nails.

B. Yeasts

By yeasts (German: Hefen, French: levures) one today understands the perfect and imperfect yeast fungi summarized in Lodders's system (1970), which form budding-cells on the one hand, but also show other morphological criteria, e. g. pseudomycelium, true mycelium, arthrospores, chlamydospores, ascospores, etc.

The number of yeast genera and species which are to be found in nature and therefore in man's environment is often quite wrongly estimated, and so a brief summary of these is given:

1. Perfect yeasts

1. Genus Citeromyces
2. Genus Coccidiascus
3. Genus Debaryomyces
4. Genus Dekkera
5. Genus Endomycopsis
6. Genus Hanseniaspora
7. Genus Hansenula
8. Genus Kluyveromyces
9. Genus Lipomyces
10. Genus Lodderomyces
11. Genus Metschnikowia
12. Genus Nadsonia
13. Genus Nematospora
14. Genus Pachysolen
15. Genus Pichia
16. Genus Saccharomyces
17. Genus Saccharomycodes
18. Genus Saccharomycopsis
19. Genus Schizosaccharomyces
20. Genus Schwanniomyces
21. Genus Wickerhamia
22. Genus Wingea

2. Imperfect yeasts

1. Genus Brettanomyces
2. Genus Candida
3. Genus Cryptococcus
4. Genus Kloeckera
5. Genus Oosporidium
6. Genus Pityrosporum
7. Genus Rhodotorula
8. Genus Schizoblastosporion
9. Genus Sterigmatomyces
10. Genus Torulopsis
11. Genus Trichosporon
12. Genus Trigonopsis

3. Yeast-like fungi belonging to the Ustilaginales

1. Genus Leucosporidium
2. Genus Rhodosporidium

4. Yeast-like fungi belonging to the Sporobolomycetaceae

1. Genus Bullera
2. Genus Sporidiobolus
3. Genus Sporobolomyces

The number of the various yeast species amount to several hundred. There are 81 different Candida species alone. Such differentiation would be too much for a routine laboratory. Therefore it is most useful to be able to use procedures requiring a limited training programme and using a reasonable amount of time and material.

Test on Rice Agar

This special medium has proved useful firstly in producing, with a high degree of certainty, the chlamydospores of Candida albicans. Secondly it allows or facilitates, in many cases, a differentiation at the genus level by evaluation of further morphological criteria such as formation of pseudomycelia, true mycelia, splitting into arthrospores, etc.

Candida albicans

This is usually the most important pathogenic Candida species, and a cause of Candidosis. The terms coined in the previous century – Oidiomycosis, Mycotorulosis and Moniliasis – have long been dropped; the occasional continued use of the term Candidiasis is wrong for two reasons: 1. the ending -iasis is used today to mean diseases caused by protozoa or vermin; 2. the stem Candid- has no -i on the end (as e. g. Monili-). This term is now, however, slowly but surely disappearing from general use.

Candida albicans can be identified morphologically and physiologically. A timesaving and elegant method of conclusively identifying it is by the definite detection of chlamydospores typical for this species.

C. Moulds and other fungi

Fungi which belong neither to the dermatophytes nor the yeasts are labelled "Moulds and others". Also in this group are the pathogens of certain "systemic mycoses" such as histoplasmosis, North American blastomycosis, South American blastomycosis (paracoccidioidomycosis), sporotrichosis, etc.

In mycological laboratory tests it is rather too ambitious to aim to recognize every mould fungus that occurs. Neither the personnel nor the facilities would be available for this. However, for practical procedure the following advice is given: a few mould fungi should be taken into the training programme, so that all those working in the laboratory know exactly what these few representatives look like macroscopically and microscopically. In the

present edition of "Laboratory Mycology" two especially common moulds are presented in such a way that they are easily recognisable as they occur in specimens in the laboratory.

1. Aspergillus fumigatus

In contrast to other moulds this withstands temperatures of 37° C and over. Therefore this fungus is the most common cause of lung mycoses in our latitude.

Occurrence of this fungus is however not limited to the mycotic lesions and is therefore not proof of aspergillosis but of course by no means precludes it. Definite identification of this fungus must be guaranteed in the laboratory.

2. Scopulariopsis brevicaulis

Formerly belonging to the penicillia, relatively common in nails, especially in old big-toe nails. Macroscopic and microscopic shapes are very characteristic so that identification is not difficult after a short period of mycological training.

For the evaluation of the pathological significance in individual cases clinical findings and microscopic appearance are important.

VII. Microscopy in mycology

Apart from the macro-morphological characteristics the micro-morphological picture is also taken into account in the detection and identification of fungi.

Under certain circumstances pathogenic fungi grow in such different forms on the skin, on mucous membrane and in the inner organs, that their appearance diverges very greatly from the culture forms and identification is difficult or impossible.

If living tissue is attacked by fungi one talks of the parasitic stage of the fungus; if the fungus is living on dead organic material then one talks of the saprophytic stage. Parallels to pathogenicity are not necessarily to be drawn from this.

Microscopic examination is applied to both stages.

A. Microscopic direct preparations

By these one understands the microscopic examination of material taken from disease manifestations. This often entails so-called fresh preparations, which are examined while still fresh, i. e. not after fixation.

1. Unstained preparations

a) Preparations with caustic potash solution

Skin scales, nail particles or hair stumps are first made very small and then placed onto the slide in 1—2 drops of 10—20 % caustic potash solution. For this one uses a dissecting needle, wire loop or mycological hook (a bent wire loop). After the coverglass has been placed on top, the preparation is carefully warmed (but not boiled) over a small flame, as hot caustic potash breaks down the keratin more quickly.

According to the density of the material to be examined, microscopic examination can be carried out at first with a low power objective and with suspected fungus threads (hyphae) with a stronger objective. In general one can only be sure of recognizing fungus threads. It is almost impossible to determine budding cells on skin, hairs and nails; therefore a culture is absolutely necessary for making sure.

On the other hand it is easier to detect budding cells in vaginal secretion, sputum, gastric juice or in smears from other areas having a mucous covering.

b) Preparations in physiological saline solution

Smears from mucous membranes and fluids such as gastric juice, spinal fluid, etc. are also examined with physiological saline solution instead of caustic potash solution (which would however not be damaging).

2. Stained preparations

a) Methylene blue staining

Yeasts in vaginal secretions may be detected when one drop of secretion is mixed with one drop of saturated methylene blue solution directly on the slide. After just 30 seconds the preparation can be examined microscopically.

Warning: It is important not to undervalue the rate of failure of this staining procedure. A high percentage of positive cases is not detected; therefore the result of the culture is absolutely necessary at the beginning of treatment.

b) Staining with Parker ink

This can be of use in routine, but is no more successful than simple caustic potash preparation, (it is however more difficult).

3. Histologic examinations

Only to be used in exceptional cases in the mycology laboratory. If required this will be discussed later.

B. Microscopic culture preparations

Examinations are carried out either through the glass side of a test-tube or by observation of a culture in a Petri dish from above. The so-called micro-cultures are especially suitable for this, and those of Ito and Refai are best. Dissected preparations or smears on the slide are further possibilities.

1. Observation of the macro-culture through the microscope

The lid of the Petri dish containing the culture is removed and the Petri dish is then placed on the stage of the microscope. Then a low power objective (e.g. 10× or 16×) is carefully moved close to the edge of a colony without letting the objective touch the surface of the fungi. As soon as a blurred picture of the edge of the colony appears in the visual field the objective is lowered with the use of the fine adjustment. Microconidia and macroconidia as well as other criteria can then be found by adjusting the focus. If the use of a more powerful objective is desired, then only microscopes with a large working space are suitable.

2. Micro-cultres according to Ito and Refai

A Petri dish containing Kimmig Agar is "windowed" in 3 or 4 places, i.e. rectangular strips of agar, ½ cm wide by 2 cm long, are cut out with a sterile scalpel (the strips are not needed afterwards). The rectangular spaces left in the agar are inoculated on 3 sides with the fungal elements to be examined and then covered by coverglasses in such a way that about ½ cm is left free in order to allow air into the area.

Under the protection of the coverglasses the fungus cultures can develop well and in this way the danger of contamination of the surface is avoided when the culture is examined under the microscope with the lid of the Petri dish removed.

There is always sufficient moisture around the free areas which can reach the micro-cultures, so that inhibition of growth through early drying out is avoided.

VIII. Therapy control

During the course of treatment of a particular case it is occasionally necessary to test microscopically and culturally the effect of the therapy and to evaluate critically the results.

A negative fresh preparation can indicate that fungus infection is no longer present or that the fungal elements are not recognisable as such. The culture will be of further help.

A positive fresh preparation gives no clear indication of whether the fungi are still alive or already dead. Clarification of this point can be obtained from the culture.

A negative culture can mean that living fungi are no longer detectable. However, the culture may also be negative because the medicaments used are sticking to the material under examination.

Positive cultures result when the daily dose has been too low or the length of treatment too short; it could also be due to resistance.

If a fungus grows which is different from that originally detected, a change of pathogen has taken place.

IX. Determination of resistance

Determination of resistance plays a much lesser role in mycology than in bacteriology. The reasons for this are many and varied. Determination of resistance is often mistakenly requested.

When is it sensible to determine resistance?

1. At the beginning of therapy: with generalized mycoses or localized organic mycoses the question of the most effective therapy must be answered as reliably as possible **before** the beginning of the treatment.

As it is usually yeasts which are concerned, the time spent — about 2 days — in determining resistance or rather sensitivity of the isolated yeasts is worthwhile, as it avoids a treatment lasting a few days or weeks with a medicament which could be ineffective. With moulds the determination of sensitivity lasts a few days longer, with dermatophytes up to 3 weeks.

2. During therapy: when resistance to therapy occurs it must be made clear whether a possible resistance of the pathogen (to the antimycotic used) could be the cause.

It is not necessary to routinely determine resistance or sensitivity to Nystatin, Natamycin/Pimaricin and Amphotericin B either in the practise or in the clinic, as truly resistant strains have not yet been found.

Similarly, determining resistance or sensitivity to Griseofulvin is only considered in rare cases, for dermatophytes which show no curling effect have not yet been found either. But on the other hand, there are dermatophytes which continue to grow well on Kimmig Agar at Griseofulvin concentrations of 1 : 10.000 to 1 : 20.000 (at higher concentrations the Griseofulvin goes crystalline in the medium); nevertheless, the infections caused by such (apparently resistant) strains of dermatophytes can be treated successfully with Griseofulvin.

There are however certain cases which do not heal when treated with Griseofulvin, although the isolated dermatophytes are quite sensitive to it. Here the reasons for resistance to therapy are to be found elsewhere.

Procedures for determining resistance

Preliminary culture: the first and particularly important prerequisite for an evaluation of resistance is the cultivation of a pure culture of the strain to be examined. It must not be a mixed culture with bacteria or other fungi. The medium for the preliminary culture (e. g. Kimmig Agar or Sabouraud Dextrose Agar) must guarantee a rich growth, for example a good formation of spores in the case of mould fungi.

Test substances: unlike bacteriology, sensitivity discs of all those substances to be considered are not yet widely used. It is therefore advisable to enquire of the manufacturers of the appropriate antimycotic to see if a test can be applied.

However, it is possible to carry out a test with either the pure substance, or therapeutic preparations. A test such as this will form a guideline and enable a judgement to be made.

Inoculation: the medium can be inoculated using a loop or mycological hook, but suspensions of fungal elements give zones of inhibition which are more easy to read. Yeast-cells, fungus spores or broken down mycelium are suspended in physiological saline solution and spread with a pipette onto a solid medium. When testing 5-Flucytosin (Ancotil) a special medium is necessary, otherwise Kimmig Agar or Sabouraud Dextrose Agar are very suitable.

After the surface has dried **the medicament is placed** on top, either as sensitivity discs, or a small amount of the pure substance, or the ready-made preparation (ointment, cream, paste, etc.). About 1 g of the preparation is best put into a hole which has been carefully stamped out of the medium with a tube, either before or after inoculation.

Reading can be made for yeasts at the earliest after 16 hours, but more usually after 1—3 days, for mould fungi after 3—8 days, and for dermatophytes after 1—3 weeks. Measurements are made of the diameters of the zones of inhibition, but an important factor when making the assessment is whether or not a zone of inhibitions has formed at all.

If no zone of inhibition is determined, then either the fungus strain is resistant to the substance being tested, or the substance does not diffuse in the medium, although the fungus may be sensitive.

X. Mycotheque

A collection of fungus strains is called a Mycotheque. This is indispensible for the purpose of comparison. More about this will be written in a later publication.

READY TO USE CULTURE MEDIA

Kimmig Agar

Selective medium for detection of dermatophytes, yeasts and moulds.

Composition:

Nutrient broth II	15.0	g
Peptone from meat	5.0	g
Dextrose	10.0	g
Glycerol	5.0	ml
Sodium chloride	5.0	g
Special agar	20.0	g
Penicillin-G sodium	40.000	I. U.
Streptomycin sulphate	0.04	g
Distilled water to	1.000.0	ml

pH 6.5

Characteristics:

The medium is yellow in colour. It is supplied in plates. For the suppression of accompanying bacteria in extremely contaminated material, penicillin and streptomycin are included.

When stored in the refrigerator the medium lasts 4 months.

Instructions for use:

The material to be examined (skin scales, hairs, nails, etc.) can be broken down first or inoculated straight onto the medium. However, as bacteria of the accompanying flora can grow to a certain extent in spite of the addition of inhibiting agents it is recommended to inoculate several plates in as many places as possible.

Incubation lasts from 5 to 30 days at room temperature and in some special cases also at 37° C. To avoid dehydration it is recommended to reseal the dishes with tape before incubation, but this should not be done hermetically as this would prevent air exchange.

Evaluation:

Dermatophytes (fungi of the genera Trichophyton, Microsporum, Epidermophyton and Keratinomyces) form a characteristic growth, as do yeasts and the most common moulds.

Selective Agar for pathogenic fungi

Selective medium for the detection of dermatophytes, yeasts and moulds.

Composition:

Soya meal peptone	10.0	g
Dextrose	10.0	g
Agar	15.5	g
Cycloheximide	0.4	g
Chloramphenicol	0.05	g
Distilled water to	1.000.0	ml

pH 6.9

Characteristics:

The medium is yellow in colour. It is supplied in plates. For suppressing accompanying microorganisms cycloheximide and chloramphenicol are added.

The medium lasts 6 month when stored in the refrigerator.

Instructions for use:

The material to be examined (skin scales, hairs, nails, etc.) can be broken down first or inoculated straight onto the medium. However, as bacteria of accompanying flora can grow to a certain extent in spite of the addition of inhibiting agents it is recommended to inoculate several plates in as many places as possible.

Incubation lasts from 5 to 30 days at room temperature and in some special cases also at 37° C. To avoid dehydration it is recommended to reseal the dishes with tape before incubation, but this should not be done hermetically as this would prevent air change.

Evaluation:

Dermatophytes (fungi of the genera Trichophyton, Microsporum, Epidermophyton and Keratinomyces) form a characteristic growth as do yeasts and the most common moulds.

Sabouraud Dextrose Agar
Sabouraud Medium

Media for the examination, isolation and storage of dermatophytes, yeasts and moulds.

Composition:

Special peptone	10.0 g
Dextrose	20.0 g
(Agar	17.0 g)
Distilled water to	1.000.0 ml
pH 5.5	

Characteristics:

The medium is light yellow in colour. Sabouraud Dextrose Agar is supplied in plates or slant tubes, Sabouraud Medium is supplied in tubes. The fast-growing bacteria are inhibited by the low pH value of 5.5. The growth of yeasts and other fungi is uninhibited.

The medium lasts 6 months when stored in the refrigerator.

Instructions for use:

The material to be examined (skin scales, hairs, nails, etc.) can be broken down first or inoculated straight onto the medium. However, as bacteria of accompanying flora can grow to a certain extent in spite of the addition of inhibiting agents it is recommended to inoculate several different plates in as many places as possible.

Incubation lasts from 5 to 30 days at room temperature and in some special cases also at 37° C. To avoid dehydration it is recommended to reseal the dishes with tape before incubation, but this should not be done hermetically as this would prevent air change.

Evaluation:

Dermatophytes (fungi of the genera Trichophyton, Microsporum, Epidermophyton and Keratinomyces) form a characteristic growth, as do yeasts and the most common moulds.

Candida Agar

modified according to Nickerson

Selective medium for detection of Candida species and other yeasts.

Composition:

Yeast extract	1.0 g
Glycine	10.0 g
Dextrose	10.0 g
Bismuth Sulphite-Indicator	8.0 g
Agar	20.0 g
Distilled water to	1.000.0 ml

Characteristics:

The medium has a milky, cloudy colour. It is supplied in plates. The medium is used for the selective growth of Candida species and other yeasts. It is presumed that bismuth ammonium citrate forms an inorganic bismuth sulphite with sodium sulphite; this complex is reduced to metallic bismuth by yeast species. This causes blackness, and in many cases a metallic shine on the cultures.

When stored in the refrigerator the medium lasts 2 months.

Instructions for use:

The material to be examined (smear from the vagina, smears from wounds, secretion from wounds, secretion from the urinary tract, urine sediment, sputum, saliva, gastric juice, tissue, etc.) is inoculated straight onto the medium.

Incubation lasts 2–5 days at 37° C and room temperature.

Evaluation:

Candida species and other yeasts have the following growth form: Medium-sized (2–3 mm), roundish, rather protruding, convex, brown-black, shiny or dull colonies.

Rice Agar

Medium used mainly for demonstrating chlamydospores and for the morphological differentiation of yeasts and dimorphic fungi.

Composition:

Infusion from rice	20.0 g
Agar	20.0 g
Distilled water to	1.000.0 ml

Characteristics:

The medium is almost transparent and has a very weak whitish-grey opalescent effect. It is used as plates and very suitable for morphological studies of Candida and other yeasts. Chlamydospores are well demonstrated.

When kept in the refrigerator the medium lasts 3 months.

Instructions for use:

Inoculation is carried out by making 3 parallel or joined streaks with a very little material of a previous culture 1 cm apart from each other, which are then covered with a sterile coverglass. Incubation lasts 24 to 48 hours at 20°–25° C and should on no account take place in the incubator at 37° C.

Evaluation:

At middle-power magnification the chlamydospores of Candida albicans which grow between the coverglass and the medium, are easily recognizable under the microscope. The pseudomycelium also develops quickly; true mycelium and arthrospores occur a little later when appropriate species (esp. Candida and Trichosporon) are in question.

ILLUSTRATIONS

Microscopic direct preparations

Skin scale from a child's head with thick clusters of hardly recognisable tiny fungus spores.

Bright field preparation

Low magnification

Hair of a child's head infected with classic microsporosis. In top right position are two air bubbles, underneath is the boundary of the fungus attack in the hair.

Higher magnification

Culture on Kimmig Agar

Typical subculture made from a primary culture which was very difficult to identify. The inoculated particles have developed into a button-shaped formation in the centre; from this furrows can be seen radiating outwards.

The surface of the culture is fine, velvety and light brown in colour; the pigment around the culture is hardly noticeable in the agar.

The culture is 2 weeks old. The aerial mycelium is still completely sterile. The formation of microconidia and macroconidia often occurs only after several weeks.

Microscopic culture preparations

Rough-walled spindle-shaped spore, pointed at both ends, only found after long searching amongst the branched aerial mycelium

Two macroconidia (spindle-shaped spores) of different form between thin mycelium threads. The septation of the spindles can be clearly seen.

Mikrosporum audouinii

Microscopic direct preparations

Skin scale with many branched fungus threads.

Phase-contrast preparation

Low magnification

Hair with a thick cuff of very small fungus-spores.

Many spores are situated around this – singly and in groups.

Culture on Kimmig Agar

A typical giant culture made up of several single colonies growing together: very fine, radial threads of yellow-orange pigment, and stronger, whitish, wooly mycelium at the points of contact between the individual colonies.

A small bunched bacteria colony can be seen at 10 o'clock sticking to the inoculated scale material.

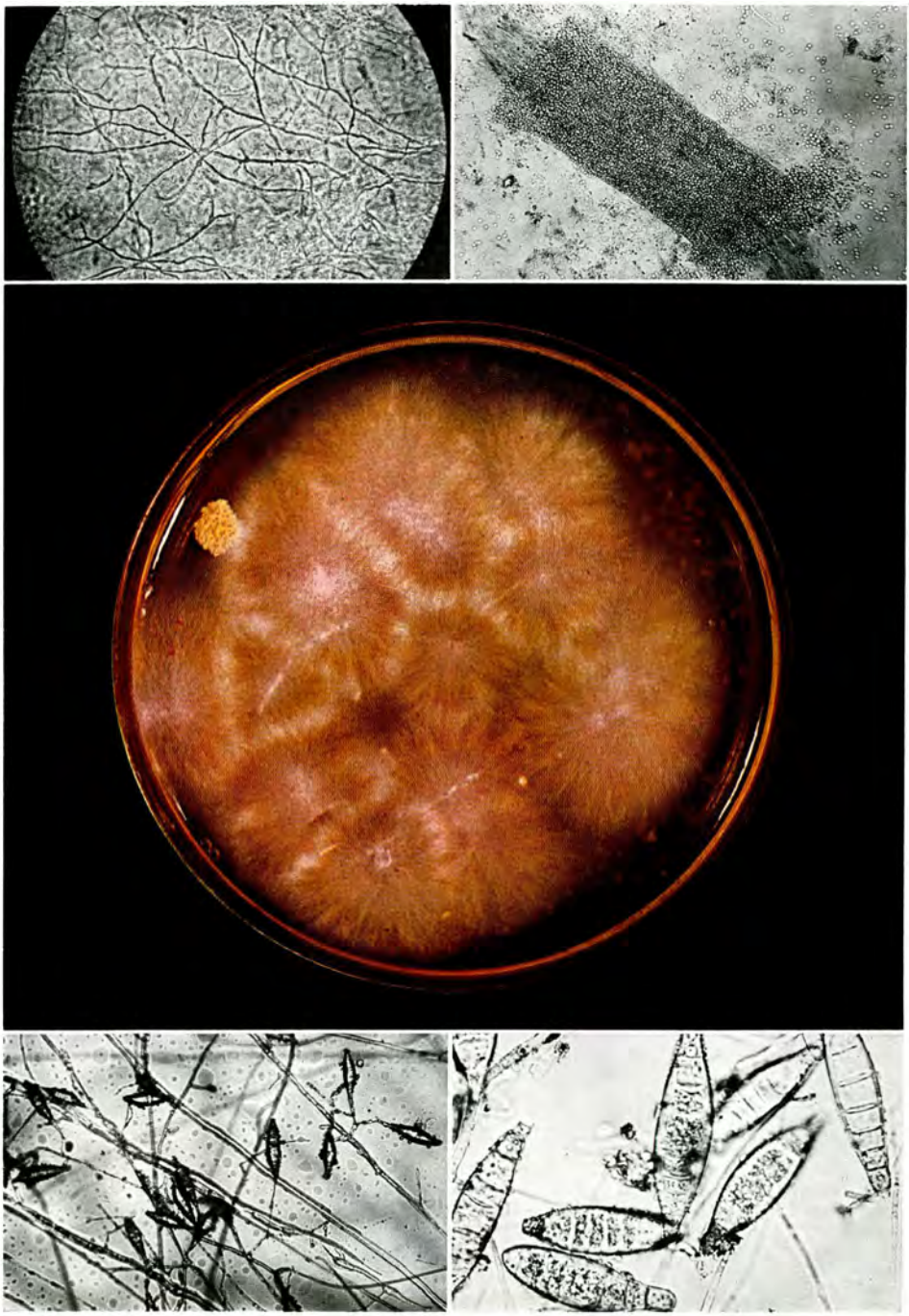
The culture is 2 weeks old. At this time the first macroconidia start to develop in the areas where the mycelium is thickest.

Microscopic culture preparations

Rough-walled spindle-shaped spores on fine, branched mycelium, grown in an Ito-Refai micro-culture

Almost smooth-walled macroconidia (spindle-shaped spores), pointed at the ends and septate in several places. Suspended in physiological NaCl-solution

Mikrosporum canis



Microscopic direct preparations

Skin scale from forearm showing extremely curved hyphae. In some places these are already divided into mycelium spores. The picture was taken one week after experimental voluntary infection.

Well cleared in caustic potash solution

Typical fungus attack of a hair by Microsporum gypseum with rather fatter spores than M. audouinii or M. canis. M. gypseum produces no fluorescence with Woodlight.

Weakly cleared in caustic potash solution

Culture on Kimmig Agar

The colony is ochre to light-brown in colour and has at the edges thin radial branches of fine mycelium network, and in the centre the beginning of a pleomorph formation. The surface is like coarse sand in texture because of the presence of large amounts of macroconidia.

The culture is 3 weeks old and is fully mature. Fertilisation was luxuriant. Only macroconidia were found in the sandy places, whereas in the slightly downy parts smaller amounts of microconidia were also found.

Microscopic culture preparations

Micro-culture in a hanging drop. Every macroconidium with 4 to 6 sections is fully germinated, however the few single-celled microconidia are not, although they are already swollen.

Bright-field photograph

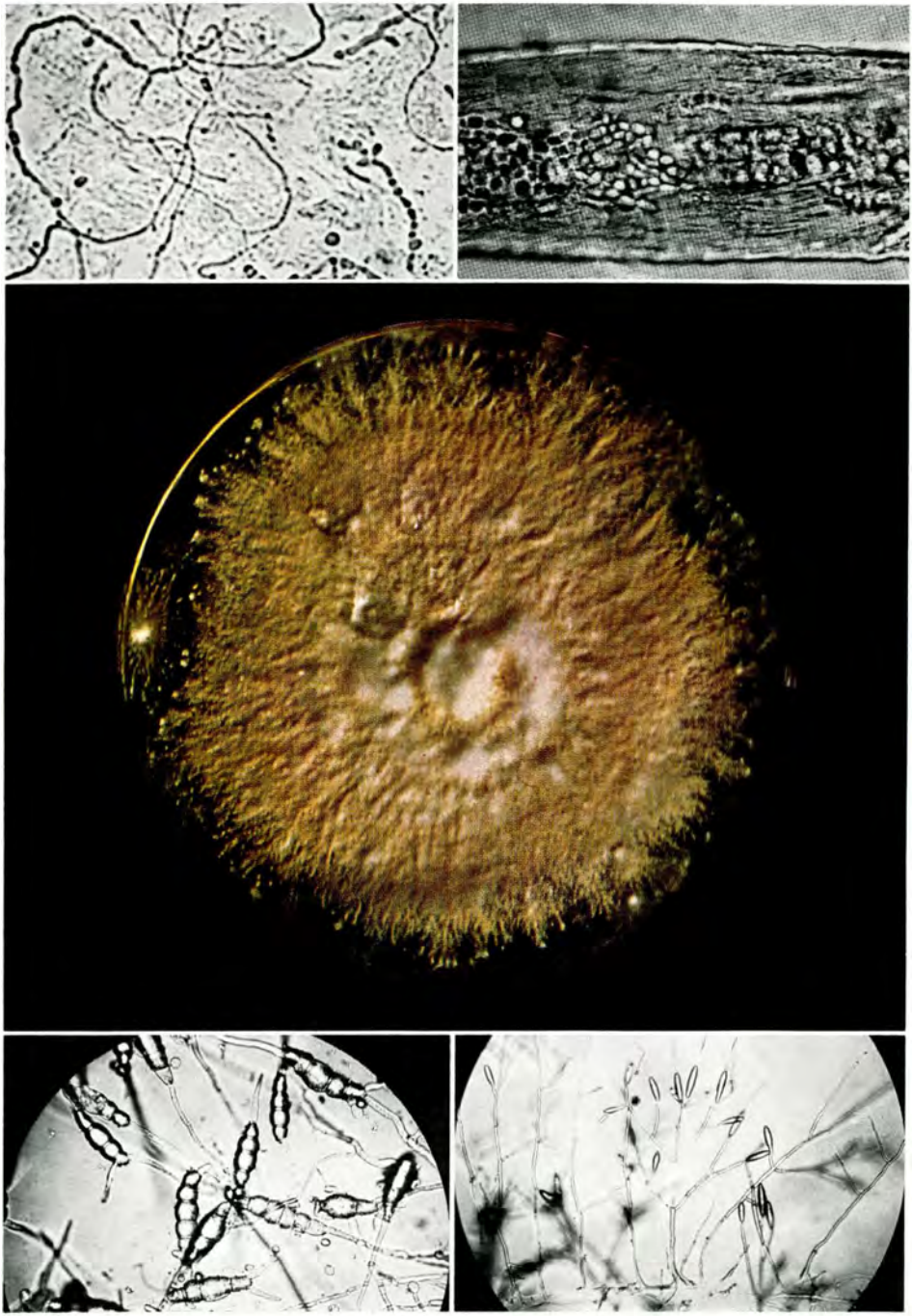
Magnified 40 fold

Micro-culture on a solid medium which can be seen at the lower edge of the picture. The "fungus plant" is growing in tree-like fashion on the medium with macroconidia on the aerial mycelium.

Bright-field photograph

Magnified 40 fold

Mikrosporum gypseum



Microscopic direct preparations

Skin scale from an area between the toes, cleared after half an hour with 15 % caustic potash solution. It has a branched vacuolated fungus thread.

Middle-power magnification (400 fold)

Hair from forearm showing fungus attack cleared after 20 minutes in 15 % caustic potash solution. It has parallel chains of mycelium spores outside and spore-free threads inside.

Middle-power magnification (400 fold)

Culture on Kimmig Agar

Culture made in triplicate by inoculating the plate in three places with material from a primary culture. These subcultures grow well when protected from air turbulence (doors and windows must be closed, air-conditioning systems must be switched off), and when material has been carefully taken from areas in the pure culture which were not contaminated.

The fine grittiness, especially at the edge, is typical, as are the almost level surface and the colour — whitish-yellow with here and there a hint of reddishness.

The culture is 2 weeks old. Fertilisation is already fully developed, but not so much so that the formation of down takes the upper hand.

Microscopic culture preparations

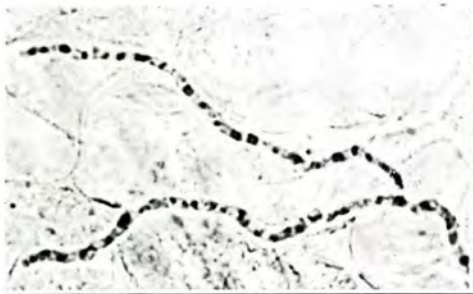
The fungus threads have changed into microconidia, of which there are many to be seen here. Microconidia formed in this way are also called aleuria.
A short spiral can be seen on the left.

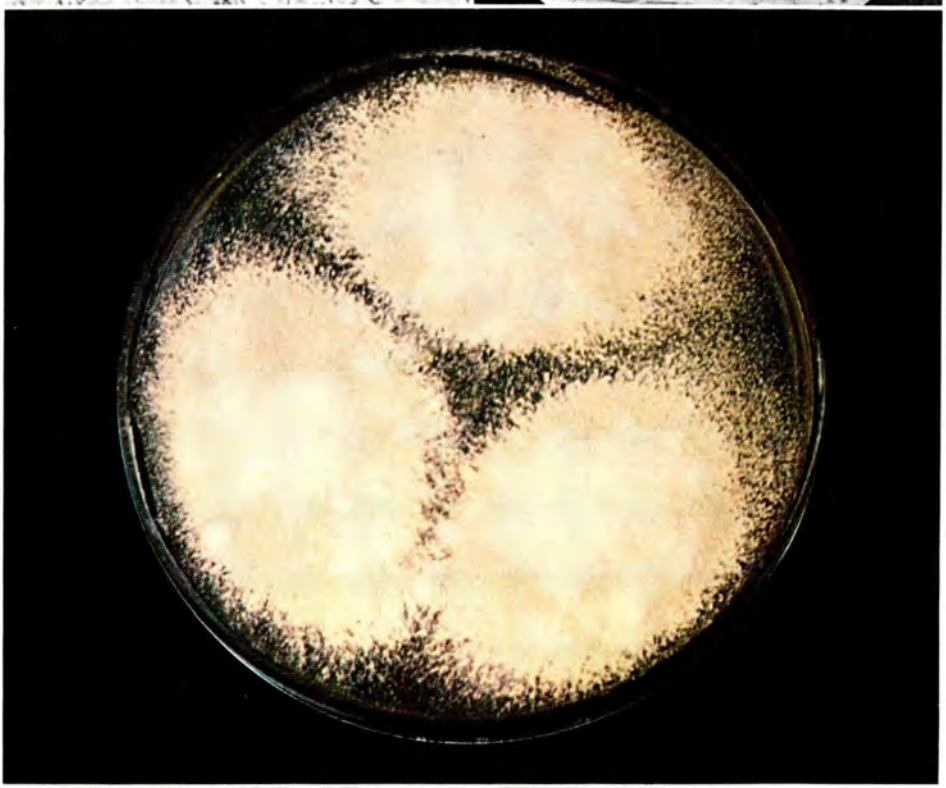
Magnified 300 fold

Five typical macroconidia which are smooth-walled, septate in several places, slightly rounded at the ends and all growing on short stalks from the same position on the aerial mycelium.

Magnified 300 fold

Trichophyton mentagrophytes





Microscopic direct preparations

Skin scale from an area between the toes, cleared in the usual way in caustic potash solution. It has isolated round cells and branched hyphae, some of which show very close septations.

Magnified 350 fold

Hair with fungus threads growing into it, cleared in caustic potash solution for slightly longer (1 hour) than usual. Some of the fungus threads have chain-like formations of mycelium spores.

Magnified 400 fold

Culture on Kimmig Agar

Six typical subcultures after inoculating material from a primary culture. Very clear button-like formation in the centre of each culture with radial furrows. The periphery of each colony shows the characteristic wine-red colour shining through the very fine mycelium.

This kind of growth-form is absolutely "diagnomonic" for the diagnosis of T. rubrum.

The culture is 4 weeks old. Microconidia have been formed very sparingly, macroconidia were not found.

Microscopic culture preparations

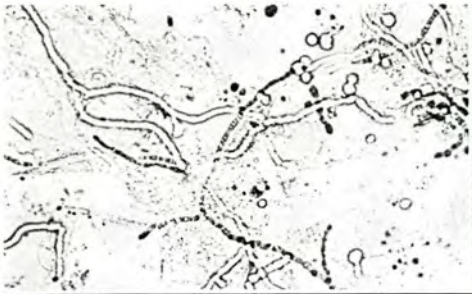
Very fine, tangled, branched fungus threads with very small microconidia (aleuria) along the hyphae which were only discovered after long searching.

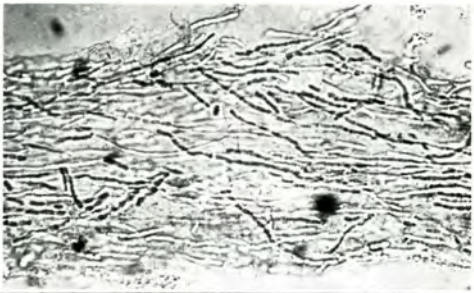
Magnified 200 fold

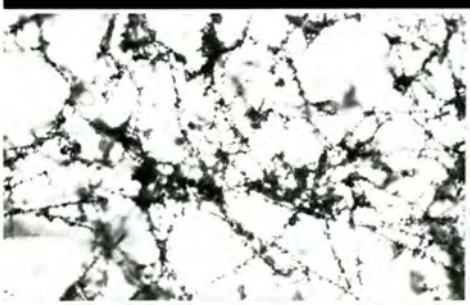
Smooth-walled, blunt-ended macroconidium divided by 3 transversal septa; next to it several unicellular, round to pear-shaped microconidia.

Magnified approx. 1000 fold

Trichophyton rubrum







Microscopic direct preparations

Skin scale from the edge of a lesion with a great number of relatively large fungus spores; also fungus threads which are finer and more difficult to recognize.

Magnified 400 fold

Hair with fungus threads growing into it (on the right hand side), partly surrounded by a thick coat of spores; this is exposed up to the middle of the picture.

Magnified 100 fold

Culture on Kimmig Agar

The pure culture is wrinkled in the centre and cerebriform with very narrow, radial furrows and crater-like dips. It has a rubber-like consistency and on the edge is a fine dust due to delicate down formation.

Around the culture the vegetative mycelium has grown deep into the medium. The colour of the culture is grey-white to ochre.

The culture is 6 weeks old. At this time no macroconidia and only sporadically microconidia were formed.

Microscopic culture preparations

Micro-culture of a fissured skin scale with the development of a branched, septate mycelium. Using this technique the results can be determined rather more quickly.

Bright-field photograph

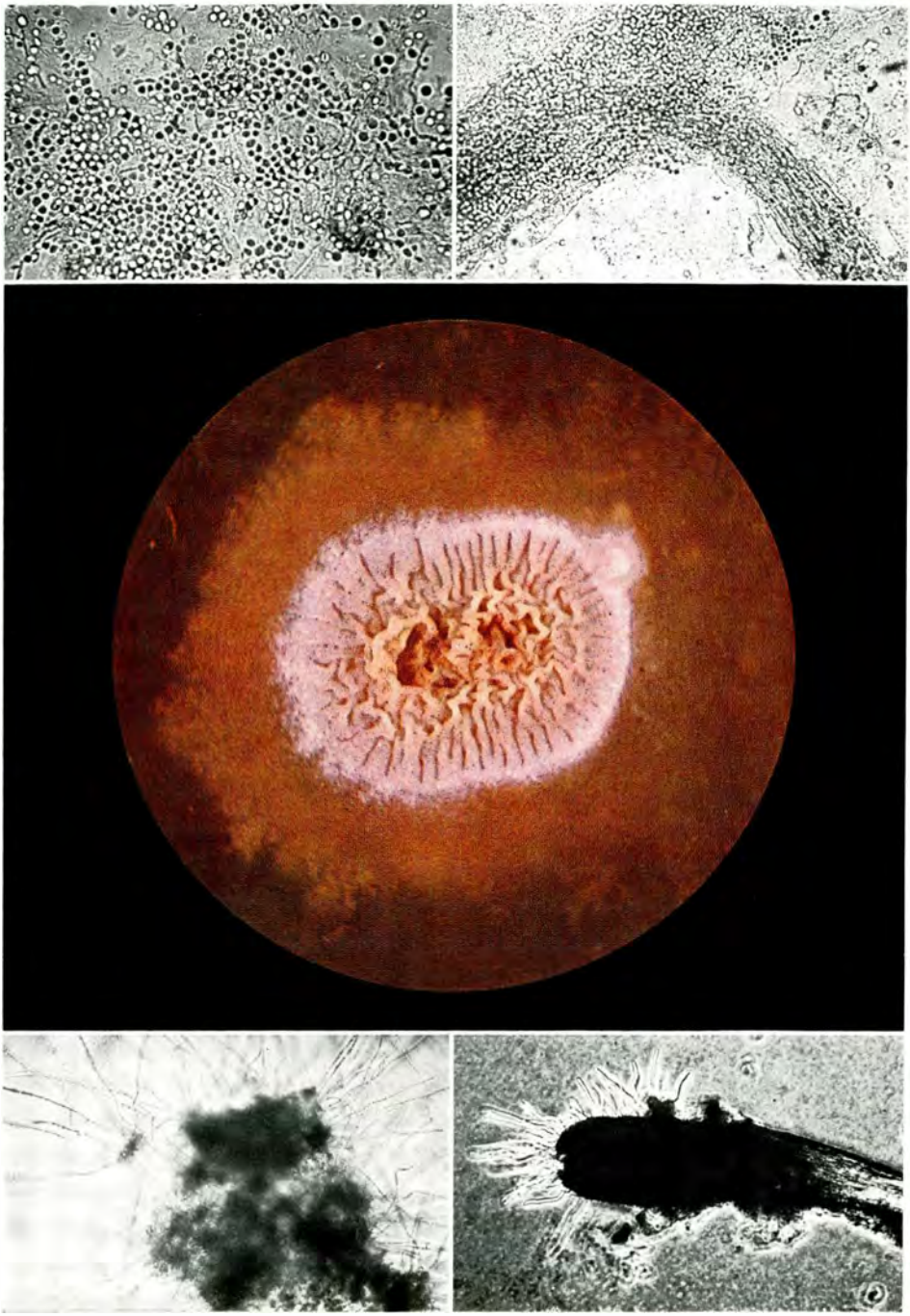
Magnified 80 fold

Micro-culture of a bed-hair or cob-hair from a child's head with fungus threads growing very slowly out of the hair. Not until after 6 weeks could the diagnosis be made.

Phase-contrast photograph

Magnified 80 fold

Trichophyton verrucosum



Microscopic direct preparations

Skin scale only very slightly cleared in caustic potash solution, with a fungus thread of many branches, and several isolated round cells (possibly fungus spores).

Hair from a dark-haired child's head. The fungus infection is especially well marked on the edge where the fungus spores are still in chain formation.

Culture on Kimmig Agar

Two very characteristic growth-form variations: the one has compact, brown-violet colonies of repressed growth and rubber-like consistency; the other has downy white colonies which grow more quickly and have luxuriant formation of microconidia and macroconidia.

The culture is 3 weeks old. The sparsely developed aerial mycelium of the dark colonies is completely sterile.

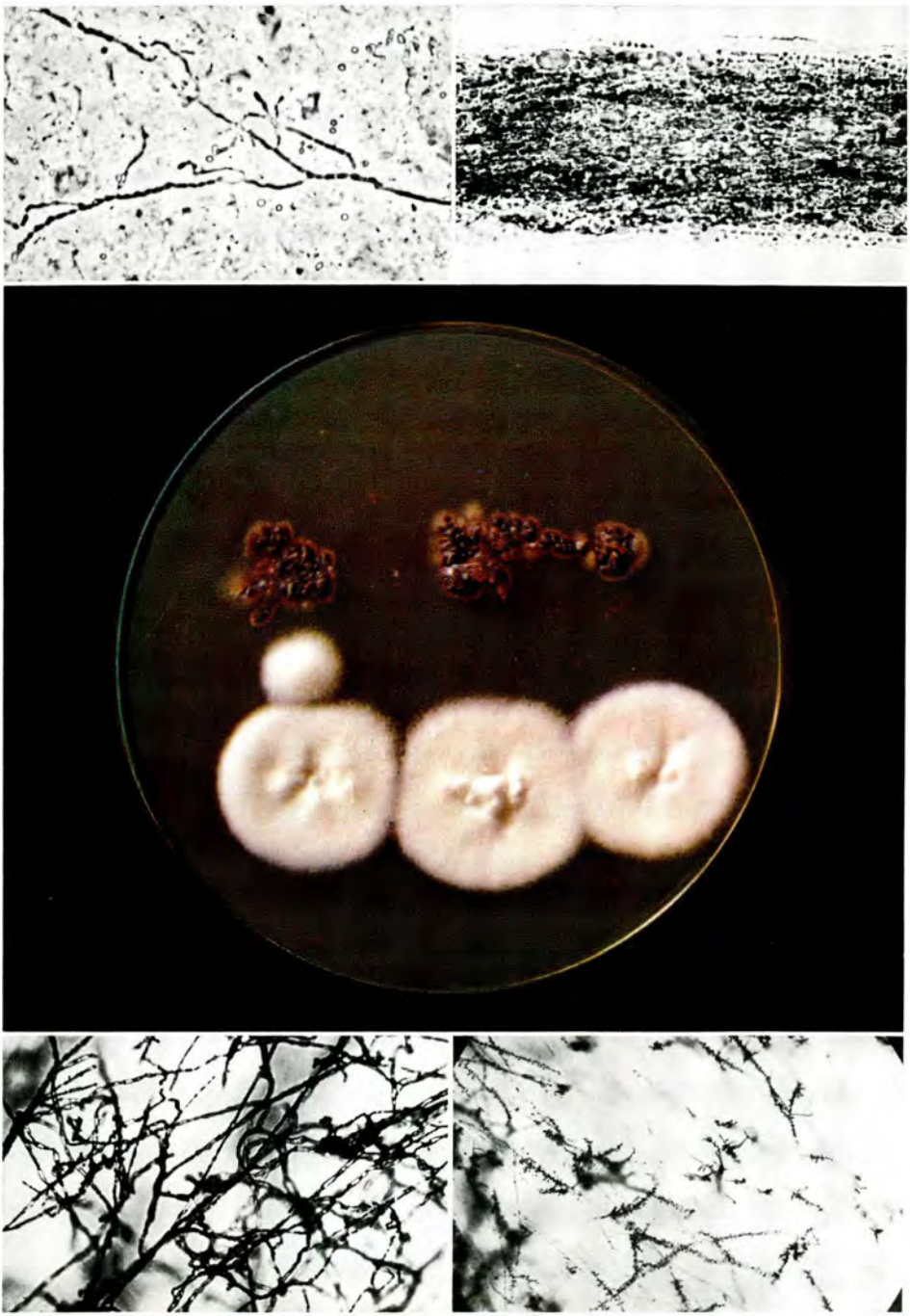
Compare also the picture on the cover which shows further variations of this extremely variable fungus. (It has altogether about 12 different variations.)

Microscopic culture preparations

Sterile mycelium with branched, septate fungus threads from the edge of a verrucous brown-violet colony three months old.

Microconidia and macroconidia from the edge of a downy-white to fine-gritty variant three months old.

Trichophyton violaceum



Microscopic direct preparations

Skin scale from thigh with a tangle of extremely branched fungus threads growing in whorls. In parts these are very narrowly septate or divided into spores.

Weakly cleared in caustic potash solution

Skin scale from the inguinal area with a very large amount of fungus threads almost totally transformed into spores at different levels of the preparation. Some of these are still very young and delicate.

Cleared more strongly in caustic potash solution

Culture on Kimmig Agar

Very typical subcultures, greyish-green in colour with slight crater formation in the centre and flat radial furrows.

The occurrence of fine, downy-white flocks, irregularly distributed over the surface of the colonies is characteristic for Epidermophyton floccosum.

The edge of the colonies is marked by the mycelium growing deep into the agar. No aerial mycelium has formed in these areas yet.

The culture is 3 weeks old. The surface had large numbers of macroconidia. Microconidia never occur with Epidermophyton floccosum.

Microscopic culture preparations

A cluster of well developed, relatively large, septate macroconidia, some of which are vacuolated, in a slide preparation, made after taking material from the centre of a culture.

Phase-contrast photograph
Magnified 300 fold

Single macroconidium from a microculture. The division into 3 cells is weakly visible. The development of septate, tube-like formations from the base cell can be clearly seen.

Bright-field photograph
Magnified 400 fold

Epidermophyton floccosum

Microscopic culture preparations

Candida albicans (on the left) with coarse pseudomycelium and terminal chlamydospores; on the right – Candida tropicalis with finer pseudomycelium and a thick cluster of blastospores.

Magnified 400 fold

Candida parapsilosis isolated from a nail-plate, with pseudomycelium and round to pear-shaped or elongated blastospores.
No chlamydospore-formation is visible.

Magnified 400 fold

Rice Agar plate

Typically inoculated cultures of 2 yeast-strains to be examined. The cultures are already 4 days old in order to allow better demonstration.

Two sterile cover-glasses per strain were placed directly after inoculation on the streaks which at this time were barely discernible. Inoculation was carried out using a wire-loop and making fine streaks with very little material from a primary culture.

Incubation took place at room temperature. The first readings are possible after just 16–24 hours.

Microscopic culture preparations

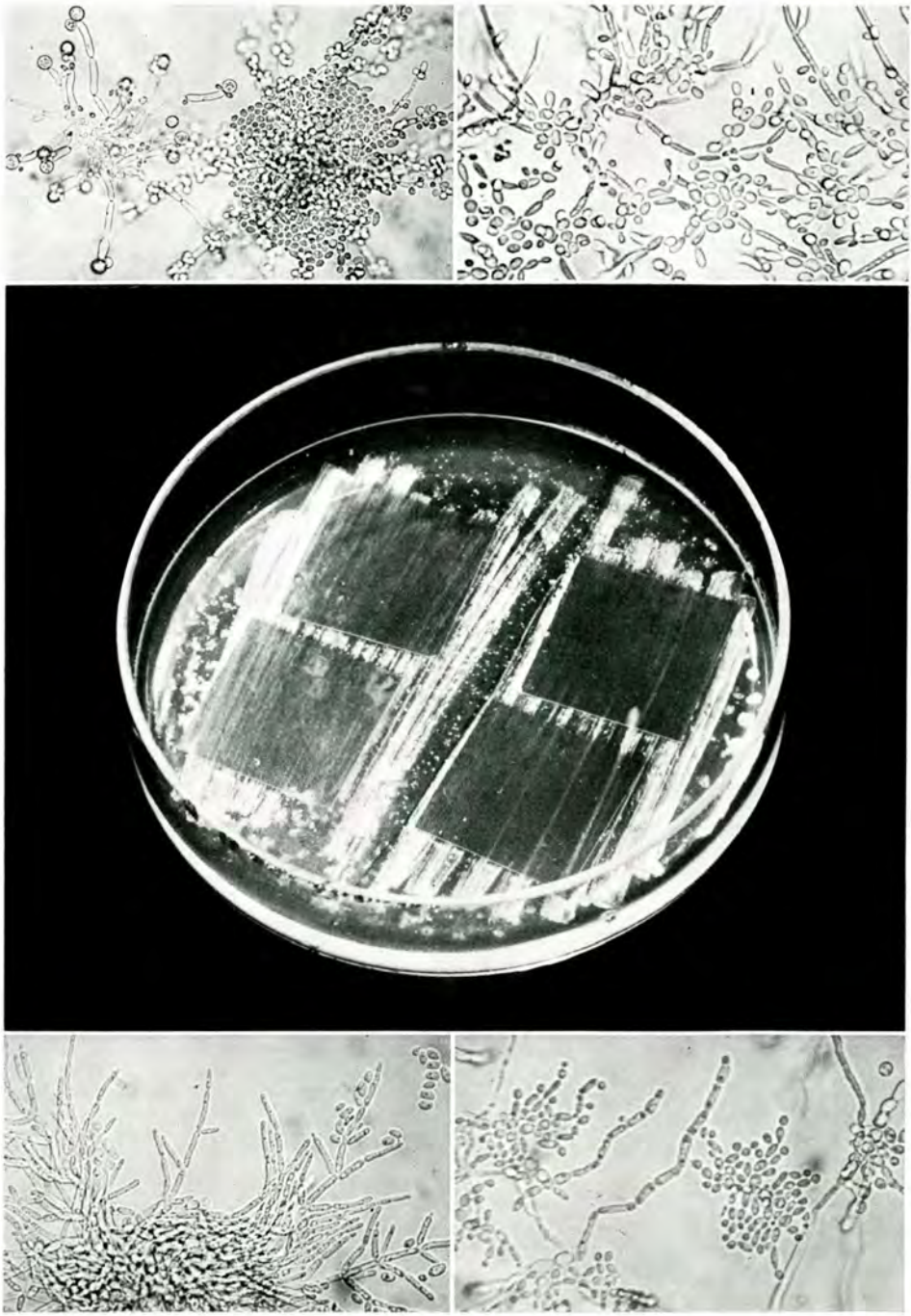
Candida krusei with characteristic longish blastospores, from which pseudomycelium has formed. Near to these, roundish to short-oval blastospores have also formed.

Magnified 400 fold

Trichosporon cutaneum showing criteria which determine the diagnosis: blastospores and true mycelium which is divided into arthrospores, often in zig-zag formation.

Magnified 400 fold

Rice agar culture



Microscopic direct preparations

Roundish budding-cells and elongated pseudomycelium from vaginal secretion. In amongst these one can see typical Doederleins rods.

Magnified 600 fold

Very fine, branched, true fungus threads (not pseudomycelium) from material taken by swab from the Glans penis. Budding-cells are nowhere to be found, but it is nevertheless Candida albicans.

Magnified 200 fold

Culture on Kimmig Agar

Creamy, compact colonies after streaking on the material with a wire-loop. Although in this case the fungus is indeed Candida albicans it must be stressed that many other Candida strains (totalling more than 80!) can look exactly the same.

Even yeasts of quite different genera, e. g. Torulopsis, are very difficult or even impossible to define macroscopically.

Important: Care must be taken when making the diagnosis. Rice Agar helps in differentiating and in determining whether it is a case of Candida albicans or not.

Microscopic culture preparations

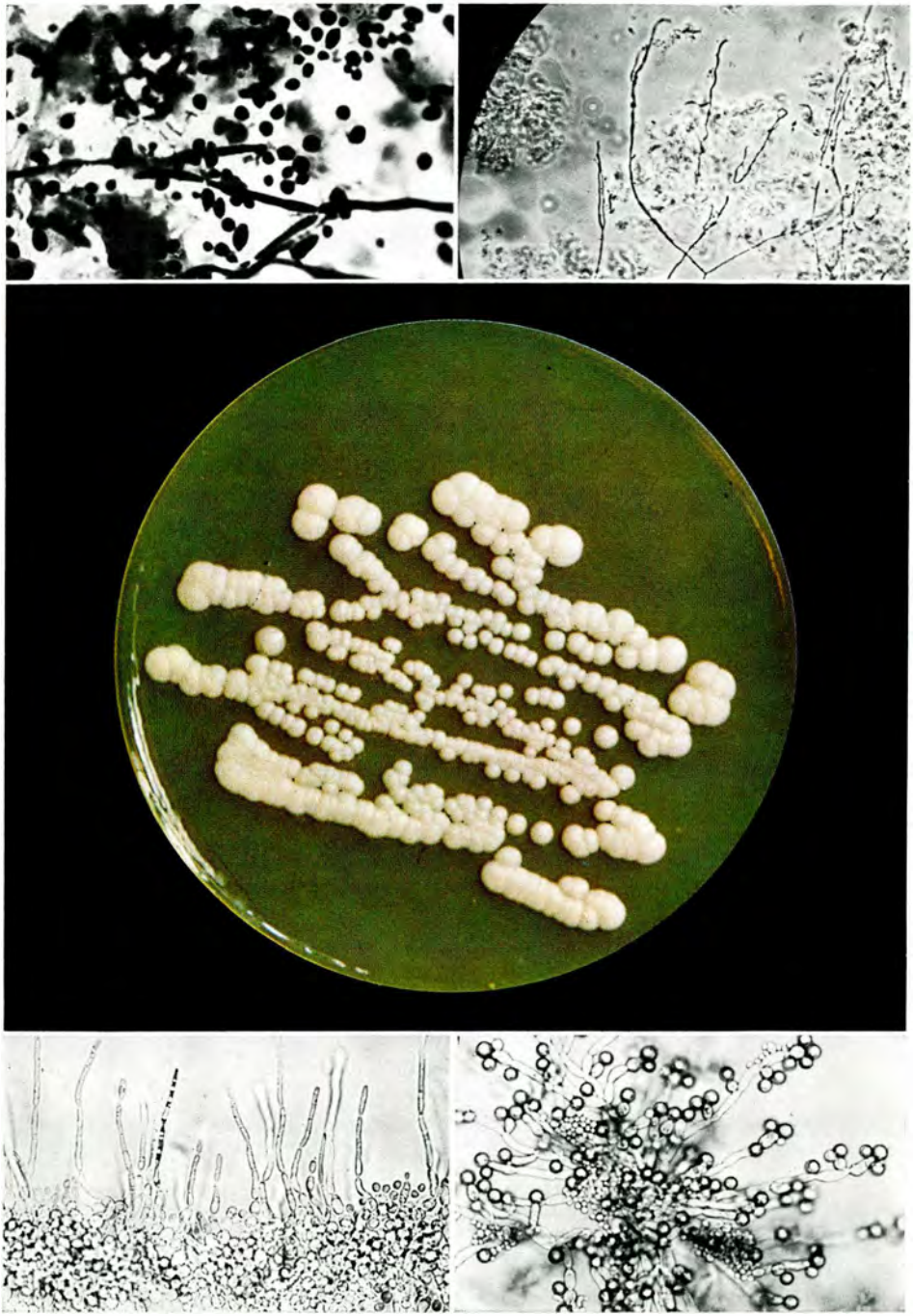
Typical picture of the edge of a micro-culture on Kimmig Agar: thick clusters of blastospores and well-developed pseudomycelium growing upwards.

Magnified 400 fold

Typical picture of Rice Agar culture: large amounts of very characteristic chlamydospores on the coarse pseudomycelium; small blastospores can also be seen.

Magnified 400 fold

Candida albicans



Microscopic direct preparations

Diffuse fungus growth in human lung tissue. Branched, septate mycelium without visible damage to the fungus.

Magnified 200 fold

Part of a small aspergilloma from a human lung. The fungus cells are greatly changed due to the defensive power of the lung-tissue.

Magnified 100 fold

Culture on Kimmig Agar

Primary culture from bronchial secretion. Bacteria have grown in the centre, around these one can see several non-confluent colonies of Aspergillus fumigatus growing on the various inoculated places on the plate. This indicates that the material was primarily interspersed with nests of mycelium and was not coincidentally contaminated with conidia from the surrounding area.

The culture is 2 weeks old. Fructification was fully developed at this point. It is to be noted that inexperienced colleagues presumed it to be a common penicillium.

Microscopic culture preparations

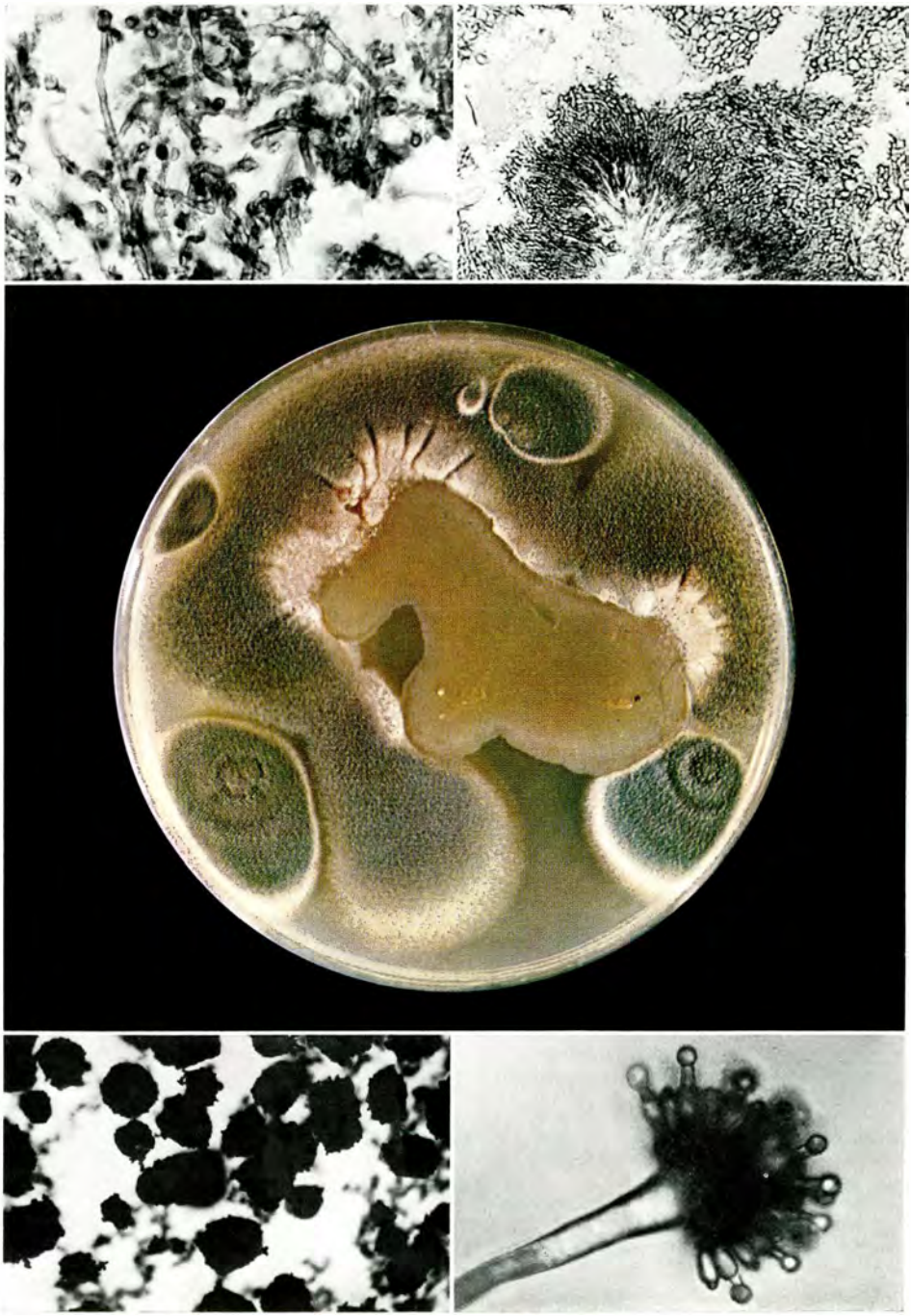
Micro-culture with numerous, compact Aspergillus heads viewed from above. The conidia chains have developed to half length and do not diverge.

Magnified 60 fold

Single conidia head with conidia starting to form on the sterigmata which are situated in a row. The conidia are typically centripetally formed.

Magnified 800 fold

Aspergillus fumigatus



Microscopic direct preparations

Skin scale from an area between the toes with a thick network of branched fungus threads some of which are very closely septate.

Bright-field photograph.

Nobbly, curved, branched, septate fungus threads in chippings from the nail of a big toe.

Phase-contrast photograph

Middle-power magnification

Culture on Kimmig Agar

Typical cultures after inoculation in rosette formation. Both the ochre to grey-brown coloured pigment and the coarse-gritty, almost sandy surface lead one to think that Scopulariopsis will be the diagnosis.

However, the diagnostic decision is made only when the microscopic criteria of the fungus have also been taken into account.

The culture is 3 weeks old. The fungus is fully developed, has used the medium exceptionally well and is waiting for favourable conditions to distribute the conidia.

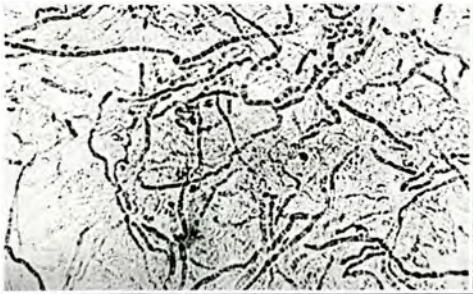
Microscopic culture preparations

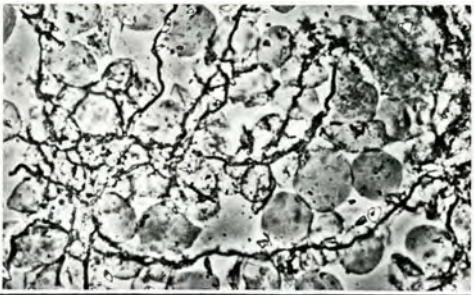
Chains of rough-walled, almost echinulate conidia on branched, septate mycelium from the edge of a colony.

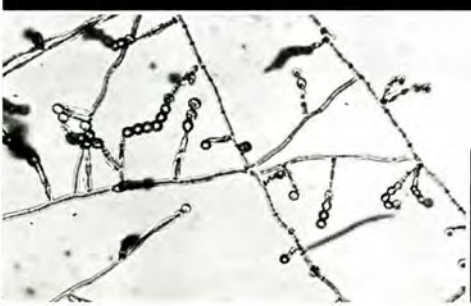
Middle-power magnification

Short chains of conidia growing out of a hair, which was experimentally infected by placing it on a culture

Scopulariopsis brevicaulis







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