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HORMODENDRUM PEDROSOI

AN ETIOLOGICAL AGENT IN CHROMOBLASTOMYCOSIS *

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Most cases of chromoblastomycosis reported from South America and Puerto Rico have been caused by the fungus classified by Brumpt in 1922 as *Hormodendrum pedrosoi.* Although this organism has been studied by several investigators, its correct classification still remains a matter of dispute. The purpose of this paper is to present evidence, based on careful comparative studies, that its correct name is *Hormodendrum pedrosoi.* Other species sometimes responsible for the disease will be discussed in a later paper.

For the purposes of this discussion, the pertinent historical facts about chromoblastomycosis can be very briefly summarized as follows. The organism was first isolated in 1911 by Pedroso from a case in Brazil.** He did not report the case until 1920[2]. The fungus did not receive a name until Brumpt's description was published in 1922[3]. In 1922 Terra, Torres, da Fonseca, and Area Leao[4] called attention to a certain type of conidiophore commonly found in cultures and transferred the fungus to the genus Acrotheca. Later, Ota[5] and Langeron[6] declared that the fungus could be placed in neither of these genera, but was a species of Trichosporium. Brumpt[7] had previously accepted this change in his 1927 edition of the Précis de Parasitologie upon the verbal recommendation of Langeron. The claims put forward for each of these affinities will be considered.

Published descriptions of *Hormodendrum pedrosoi* are not satisfactory because of varying degrees of incompleteness, misplaced emphasis on certain morphologic features, or false

* Received for publication January 21, 1936.

** A critical review of all cases reported up to 1933 is given by MacKinnon.[1]

interpretations. At the cost of some repetition, therefore, the growth habit and morphologic features of this fungus are here given so that we may have a sound basis for a discussion of its correct classification. The strains studied include those isolated from Puerto Rican cases, old stock strains from South America, three recently isolated strains which Dr. da Fonseca very kindly sent us, one strain from the Centraalbureau voor Schimmelcultures, Baarn, and one strain of *H. algeriensis*. All of these are similar except for certain minor differences which seem in part to be correlated with the time intervening since the strain was isolated. Three strains of *Phialophora verrucosa,* one of *H. langeroni,* and saprophytic species of Hormodendrum, Acrotheca, and Trichosporium were used for comparative studies.

CULTURES

In culture, *Hormodendrum pedrosoi* grows more slowly than the common saprophytic species of the genus. On Czapek's agar the colony reaches a diameter of only a few millimeters in a week, and the aerial hyphae consist mostly of conidiophores, so that the surface appears dusty. The culture is greenish gray to olive. As the colony on this medium becomes older it remains flat, with sparse aerial growth and assumes a darker olivaceous or olive-black color. The margin is, in most strains, uneven, often showing extensive arborescent extension into the surrounding agar.

On Sabouraud's maltose agar the growth is somewhat more rapid and there is a more profuse development of aerial hyphae. After 2 weeks of growth at room temperature on this medium the colony is about 15 mm. in diameter and 6–7 mm. high. When seen from above, two zones are apparent, a central hemispherical zone and a gently sloping peripheral zone. When viewed from the side the colony appears dome-shaped, the angle between the two zones being filled out by delicate, grayish, aerial hyphae (figs. 1 and 2).

After 4 weeks at room temperature on Sabouraud's maltose agar a colony reaches a diameter of about 45 mm. and a height of 7–8 mm. At this time the colony has the shape of a low cone with a nearly even slope, although some cultures are almost flat. The margin of the colony is even and composed of short ascending aerial hyphae, there being no extension of hyphae in the substrate beyond this zone. The surface is covered with short, delicate, erect or ascending, grayish, aerial hyphae. There is a conspicuous zonation apparent in many cultures of some strains. A typical colony shows, at the center, an olive black zone 5 mm. in diameter, and surrounding this appear successively a 6 mm. zone of brownish olive, a 1 mm. zone of olive black, a 2 mm. zone of brownish olive, a 1 mm. zone of olive black, and a peripheral zone of 8–10 mm. of olive gray fading into gray at the margin. In older cultures there may be a narrow zone of bluish green near the margin of the colony (figs. 3 and 4).

In some strains the zonation is not so marked, and there may be other color differences. In general, the color is mostly due to the underlying mycelial mat which exhibits various densities of olive and olive black, and is overgrown by short, delicate, gray, aerial hyphae. The color is also modified by the degree of sporulation reached in the culture. Richly sporulating cultures develop more of the brown and olive shades with a dusty surface, while those with relatively low production of conidia tend towards gray and have a velvety surface. In some strains, islands of more or less sterile growth appear near the center of some cultures (fig. 4.) Cultures on Sabouraud's maltose agar after two or three months are brown.

SPORE FORMATION

The microscopic appearance of the fungus is that of a Hormodendrum. It differs from the more common saprophytic species of the genus by its slower growth and shorter chains of conidia. The conidiophore of *H. pedrosoi* is an erect or ascending branch arising from the vegetative mycelium and differentiated from it by a somewhat deeper pigmentation (fig. 9). The conidiophore may bear branches (fig. 5), or it may be unbranched (figs. 9–14). The tree-like appearance of a well developed spore head is due to these branches of the conidiophore, when present, and to branching conidial chains.

There are five general types of spore head in *H. pedrosoi,* but all intermediate types occur so that they can not be sharply differentiated.

In the simplest type of spore head the conidiophore bears at its tip a cluster of spores (fig. 10). Older conidiophores may bear spores not only at the tip but on the sides for some distance below the tip (fig. 11); these constitute the second or Acrotheca-like type of conidiophore. In the third group the spores first formed at the tip enlarge and elongate (sometimes so much as to resemble hyphal branches) and bear at their tips secondary spores (figs. 12–13). Development may even proceed to a more complex stage with the production of tertiary spores (fig. 27). In the fourth group the arrangement is less regular, and spores are borne in branching conidial chains (figs. 14–15). We may consider as belonging to a fifth type of spore head those in which there is a combination of some or all of the other types (figs. 28, 17). In most healthy cultures this type predominates. A conidiophore may bear one or more simple spores which have not developed further, together with modified conidia bearing secondary verticils, and others bearing more or less complex branching conidial chains (fig. 6). The first two types are, we believe, depauperate types; the last two represent the characteristic method of sporulation. A normal spore head of each of the five types described begins as a crown of spores at the tip of the conidiophore. The degree of complexity reached depends upon the subsequent development. One, or more, or all of these primary spores may bear secondary conidia or branching conidial chains. Saprophytic species of Hormodendrum undergo precisely the same stages of development. They differ only in the greater length of conidial chains.

The spores of *H. pedrosoi* are olivaceous under the microscope, smooth-walled, and they vary greatly in size and shape, depending upon their position toward the base or the tip of the conidial chains. Those at the base of a mature chain are $2.5–3.5 \times 7–10\ \mu$, exceptionally reaching a length of $13\ \mu$ or even longer; those toward the tip are $1.5–3 \times 3–5\ \mu$. The young spore arises as a thin-walled hyaline bud which becomes thick-walled and pigmented and assumes an oval shape with a narrowed base at the point of attachment (fig. 19). The spore head is easily dissociated, and if this be done the detached spores are found to vary considerably in shape (fig. 29). Those from the base of a chain have the outline of an elongated shield, with a single apiculus at the base marking the point of attachment to the conidiophore (or to another spore if its position were not basal in the spore chain), and as many as four apiculi at the upper end upon which spores or spore chains have been borne (fig. 29). Many of the spores are widened upward to accommodate the secondary spores which they have borne. If a spore has given rise to only two secondary spores its shape is less strikingly modified, and if it has borne only one spore, instead of giving origin to a branch in the conidial chain, the two ends of the spore are similar (fig. 29). All these diverse conidial forms are common to *H. pedrosoi* and the saprophytic species of the genus (fig. 24), and in both it is possible to find spore chains of varying length in which the elements are but little modified in shape. In cultures of *H. pedrosoi* in which branching spore heads have not been numerous there is a corresponding decrease in the number of modified spores and an increase in the relative number of terminal spores with rounded tip and pointed base.

DISJUNCTORS

Langeron [6] objects to the admission of the fungus to the genus Hormodendrum because he does not find disjunctors on its spores. The so-called disjunctor of Hormodendrum is nothing more than the narrowed end of the spore and the thickened wall which surrounds and terminates it. The thickened walls over these pointed tips are less deeply pigmented than the rest of the spore wall, and appear bluish or refringent under the microscope. They have somewhat the appearance of being gelatinized. This modification, whatever it is, apparently contributes to the easy dissociation of the spores when mounted in a liquid medium, but is much less highly differentiated than the disjunctors of Sclerotinia and Albugo, for example.

The spores of all the Brazilian, as well as the Puerto Rican strains we have examined, exhibit this modification (figs. 19–22). For comparison the corresponding structures in a saprophytic species of the genus are shown in figures 23–24. In both *H. pedrosoi* and the saprophytic species of Hormodendrum there is exactly the same type of modification.

In most spores of *H. pedrosoi* it is not so conspicuous as in most spores of the saprophytic species, but the difference is one of degree and not of kind. On the other hand, it is sometimes well developed in *H. pedrosoi* (figs. 21 and 22). While it is not a diagnostic character of fundamental importance, its presence in this pathogen corroborates our belief that the fungus is a species of Hormodendrum.

DISCUSSION

Admission of this species to the genus Hormodendrum depends upon the interpretation of the subterminal elements in a spore head. Some investigators, basing their judgement apparently upon the morphologic appearance of the mature form, have pronounced these subterminal structures as elements of the conidiophore, and have claimed that only the terminal cells are spores. Diverse conidial elements are to be found, not only in *Hormodendrum pedrosoi,* but also in any common saprophytic species of Hormodendrum (fig. 24). That all are true spores is indicated by their manner of development. By repeated observations of a given conidiophore as it grows in a small culture cell it is possible to follow the development of a given cell from its first appearance as a tiny bud, then as a spore, until it becomes modified in size and shape with the production of secondary spores. The cells which grow from the tip of a conidiophore originate and develop as spores. When mature cells of this type are sown upon agar they germinate, not in the manner of broken hyphal elements, but as spores.

In a previous publication [8] comparing spore formation in Trichophyton and Hormodendrum it was pointed out that there is a fundamental difference between spore production in forms in which the youngest spore is at the tip of a chain, and in such forms as Penicillium, where the conidiophore is highly specialized and the youngest spore is always at the base of the conidial chain. In the latter type the mature spores tend to be uniform. In the indeterminate type of sporulation as it appears in Hormodendrum there is necessarily some modification in the subterminal spores. They are apt to be larger than the terminal spores, and they are usually more or less deformed to accommodate the secondary spores which they bear (fig. 29). The statement that only the terminal cell of these specialized chains of cells is a spore is not tenable. One who tries to maintain this thesis would be compelled to specify also the time at which his diagnosis is made, because the cell which is by his definition a spore today, will tomorrow, again by his definition, be an element of the conidiophore. In spore heads grown under optimum conditions spore chains may reach a length of several cells (figs. 9 and 28). *Such fructifications are in all respects those* of a Hormodendrum. If *H. pedrosoi* be excluded from the genus, so also must the common saprophytic species of the genus, and this, obviously, would be absurd. The production of these chains of conidia makes it impossible to classify the fungus as a species of Acrotheca or Trichosporium.

PHIALOPHORA SPORE TYPE

Besides the Hormodendrum type of spore, which is the characteristic and strongly predominant type in this species, a second spore form is occasionally found. As previously reported [9], we have recently found conidiophores and conidia of the type long known to be characteristic for *P. verrucosa* in all strains of *H. pedrosoi* examined and in *H. compactum.* They were found also in a saprophytic species of Hormodendrum [10].

The Phialophora type of conidiophore is to be found in old cultures of *Hormodendrum pedrosoi* and never in large numbers. It is best seen in slide cultures on corn meal agar. It may be closely associated with a Hormodendrum spore head, arising even through the transformation of a spore (fig. 26), or it may be a solitary, well differentiated conidiophore more closely resembling those of *P. verrucosa* (figs. 7–8). This type of conidiophore need never be confused with a broken hypha, even when the latter contains lipoid granules, differentiation being marked by a thinner wall and a flaring mouth to the cup. These broken hyphae may be numerous in teased mounts from a tube or plate culture; they are rarely seen in a properly prepared slide culture. The broken hypha is cylindrical and its wall terminates abruptly at the break. The irregularly broken wall can be clearly seen, and instead of flaring it may be somewhat constricted at the point of breaking.

The Phialophora type of conidiophore, on the contrary, is flask- or bottle-shaped. It may or may not be inflated toward the middle (fig. 7), but at the point where the spore is formed it is constricted. Above this point the wall flares to form a cup, the rim of which is thin (fig. 8) and very different in appearance from the wall of normal thickness which terminates a broken hypha. Within this cup the conidia are budded out successively from the base (figs. 7–8). In rare cases the spore is borne excentrically.

Measurements for the single celled conidiophores vary from 6 μ to 12 μ in length and from 2 μ to 3 μ in width. In cases where the conidiophore terminates a hypha, it is usually the terminal cell only which is modified and it seems best to give measurements for this unit. The conidia have thin walls and are oval or egg-shaped. There is considerable variation in size and in agar cultures there is an increase in size after the spore is discharged. Dimensions are 1.5 μ–2 μ by 2 μ–3 μ.

The Phialophora spores are rare in *H. pedrosoi,* but their rarity is no index to the importance of this discovery. The occasional production of this type of conidia in *H. pedrosoi, H. compactum,* and in some saprophytic species of Hormodendrum, as well as in *P. verrucosa,* proves a close relationship among the three fungi which cause chromoblastomycosis. The finding of this type of spore in a saprophytic species of Hormodendrum and in *H. pedrosoi* supplies, moreover, further proof that the latter is correctly placed in the genus Hormodendrum. Widely divergent in morphology as the fungi of chromoblastomycosis have come to be, we are led to believe, on this and other grounds, that they are phylogenetically related to each other and to some saprophyte or saprophytes of the genus Hormodendrum. Data regarding this type of spore and the taxonomic problems involved are discussed more at length in an accompanying paper.

In addition to these spore forms we have observed in old cultures of *H. pedrosoi,* masses of pigmented, pseudoparenchymatous cells (fig. 18) which resemble those of the parasitic phase of the fungus in tissue. Their significance is not understood.

ANIMAL INOCULATIONS

Animal inoculations also indicate a close relationship between *Hormodendrum pedrosoi* and some saprophytic species of Hormodendrum. It is well known that characteristic lesions are produced in rats inoculated with *H. pedrosoi.* Rats were inoculated with saprophytic species of Hormodendrum and later killed and examined for evidence of infection. Investigation of this point seemed of interest because *H. pedrosoi* is presumably a wound parasite. None of the saprophytic species used were virulent parasites, but in rats killed six weeks after inoculation there was some evidence of proliferation of the fungus cells used as inoculum. These fungus cells resembled those found in lesions of chromoblastomycosis, and they had elicited the same tissue reaction as the pathogenic species. *H. elatum* was recovered in culture from a small lesion at the point of inoculation in a rat inoculated 3 months previously.

CLASSIFICATION

With the foregoing description of the fungus in mind let us examine the reasons given for the different names under which it has been known. The first name applied to this fungus was *Hormodendrum pedrosoi* given by Brumpt in 1922, and with this classification we agree for the reasons given above.

It is easy to see, however, why da Fonseca and his associates changed the name of this fungus to *Acrotheca pedrosoi.* It is true that many of the conidiophores one sees resemble those of species of Acrotheca. They are septate, dark, and bear conidia in acro-pleurogenous arrangement. The conidia are pigmented and some of them are of suitable shape, although they are much smaller than those of most species of Acrotheca. The points of insertion of these conidia are marked by protuberances on the conidiophore as in Acrotheca. This type of fertile structure, however, is not the most highly developed type produced by the fungus, and therefore does not determine the genus. Examination of a culture will reveal the presence of all transitional stages between an imperfect spore head of this type and the fully developed conidiophore, bearing branching chains of conidia characteristic of species of Hormodendrum. Even when this latter type of fertile structure is produced in a culture its presence may be overlooked because its elements are so easily dissociated. When material is mounted under a cover slip for examination, unless skillfully done, few spores will be found in place upon such a conidiophore. Branching chains of conidia can be observed, however, when a plate culture is examined under the microscope, or when the fungus is studied in a slide culture or a small culture cell. These branching chains of conidia may be short, but in other cases they can reach a length of several cells (fig. 25). Their production definitely excludes this fungus from the genus Acrotheca. While development of catenate spores is the salient point differentiating this fungus from Acrotheca, which never bears spores in chains, confirmatory evidence is supplied by the general habit of growth of the fungus, the character of its spores, and, finally by its obvious affinities with the genus Hormodendrum.

The claims made for the name Trichosporium * are more easily disposed of. In this genus the spores are borne in clusters (not in chains) at the tips of poorly differentiated conidiophores (Fries, Saccardo, Costantin, Langeron). The fungus of chromoblastomycosis does not belong in this group for the fundamental reason that it produces chains of spores. Furthermore, if it were true that the terminal elements only of a spore head of *Hormodendrum pedrosoi* are spores, we must admit that the conidiophores which bear them are

* Langeron's rejection of the genus Acrotheca for this fungus is based upon a misinterpretation of the brief descriptions and key characters of the genus. His rejection is based upon the statement that the vegetative mycelium in Acrotheca may be suppressed or even lacking, and that the conidiophore, on the contrary, is 'well developed and differentiated. It must be remembered that these early descriptions of the saprophytic fungi and the characters given in the keys to Saccardo are based in most cases upon the development of the fungus in nature. The description cited merely means that when the fungus is found on its natural substrate, usually of decaying plant material, there is no conspicuous aerial development of vegetative mycelium. The visible part of the growth consists chiefly of conidiophores, while the vegetative mycelium is submerged in the substrate. When species of Acrotheca are isolated in pure cultures and grown in test tubes their manner of growth is altered and they produce abundant aerial vegetative mycelium. The fungus of chromoblastomycosis can not be placed in the genus Acrotheca for the reasons given above, but its exclusion can not be based on its production of a vegetative mycelium.

indeed very high specialized structures (figs. 9, 27–28). In either case the fungus can not be included in the genus Trichosporium.

SUMMARY

The fungus causing most cases of chromoblastomycosis in South America and Puerto Rico is a species of Hormodendrum and its correct name is *H. pedrosoi* Brumpt 1922. (Syn. *Acrotheca pedrosoi, Trichosporium pedrosoi, T. pedrosianum*).

Its affinities with the saprophytic species of the genus Hormodendrum are shown by its similar but slower growth habit, its pigmentation and, most important, its production of branching chains of spores of the Hormodendrum type.

The subterminal elements of these chains are true spores and not elements of the conidiophore. This is shown by their manner of development, transitional forms, and ready germination in the manner of spores.

The fungus can not be admitted to either Acrotheca or Trichosporium, in neither of which genera are the spores borne in chains.

The so-called disjunctors are present in *H. pedrosoi,* as well as in saprophytic species of the genus. Furthermore, it has in common with at least one saprophytic species of that genus, occasional conidiophores and conidia of the sort previously described for *Phialophora verrucosa.*

Successful animal inoculations with saprophytic species of Hormodendrum add corroborative evidence for a close relationship between the pathogenic and the saprophytic species.

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PLATE 1

FIGS. 1–2. Cultures of two strains of *Hormodendrum pedrosoi* two weeks old on Sabouraud's maltose agar.

GRABADOS 1 y 2. *Colonias dos razas de* Hormodemdrum pedrosoi, *en agar maltosado de Sabouraud a las dos semanas de la siembra.*

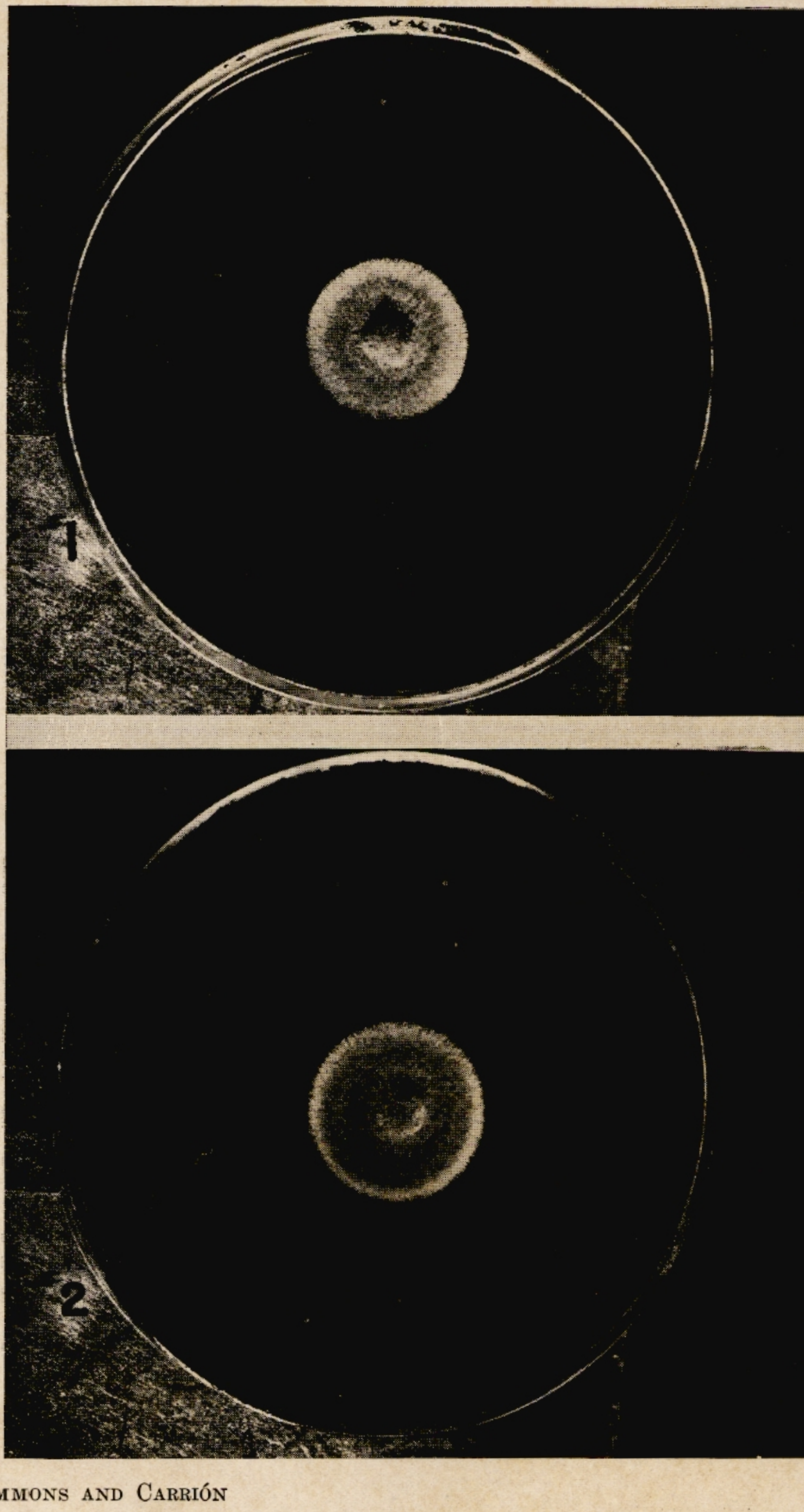

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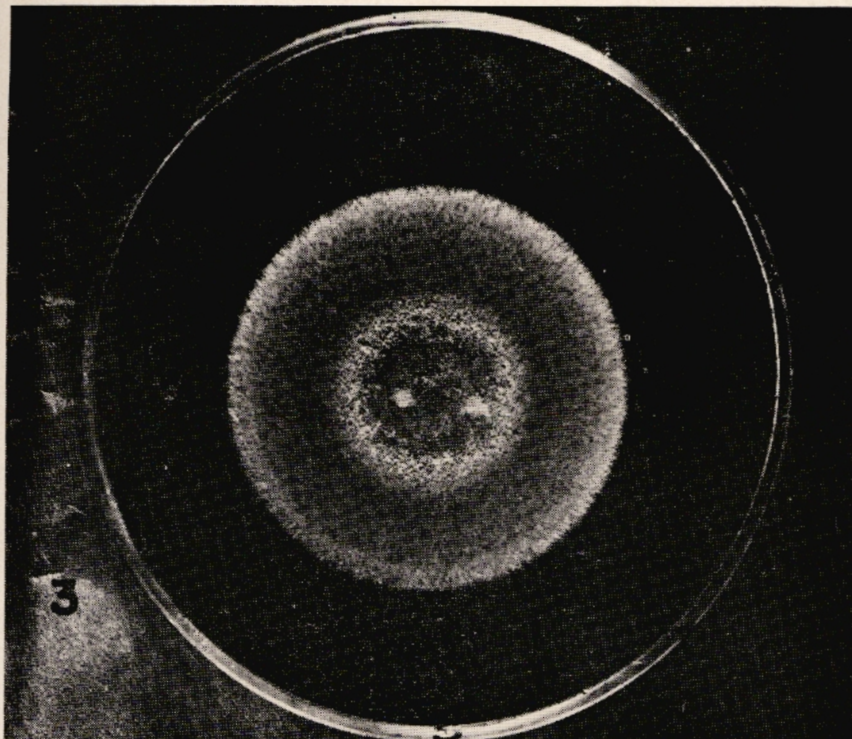
PLATE 2

FIGS. 3–4. Cultures of two strains of *H. pedrosoi* four weeks old on Sabouraud's maltose agar.

GRABADOS 3 y 4. *Colonias de dos razas de* H. pedrosoi *en Sabouraud, a las cuatro semanas de la siembra.*



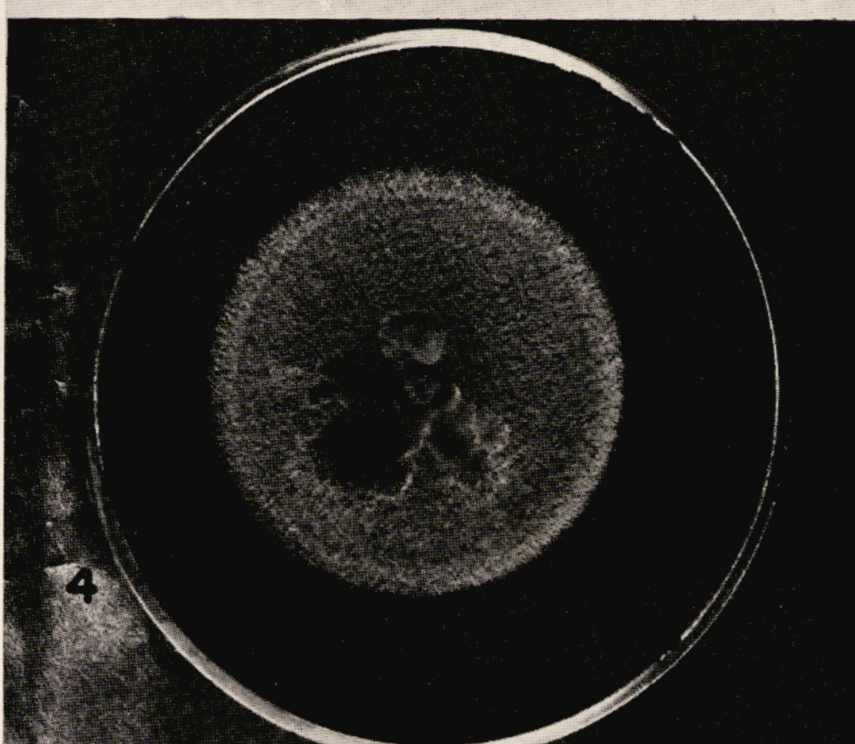


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PLATE 3

Figs. 5–6. Photomicrographs of conidiophores and conidia of *H. pedrosoi* from corn meal agar slide cultures. × 1000.

Figs. 7–8. Conidiophores of the Phialophora type in *H. pedrosoi*. The arrows indicate cups containing spores. × 1000.

Grabados 5 y 6. *Microfotografías de conidióforos y conidios de* H. pedrosoi *en un cultivo de agar-maíz* (× 1000).

Grabados 7 y 8. *Conidióforos de tipo Phialophora en el* H. pedrosoi. *Las flechas señalan los cálices que contienen los esporos* (× 1000).

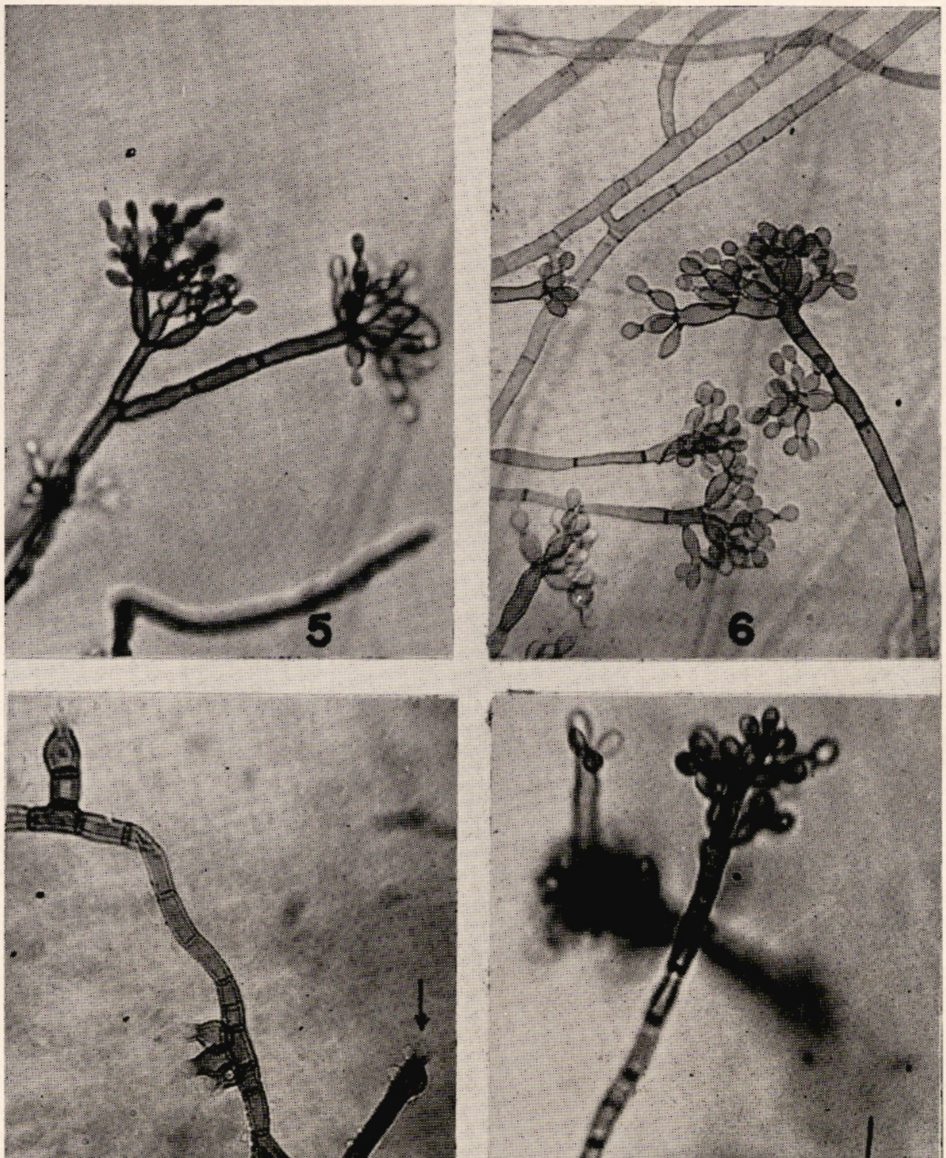

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PLATE 4

FIGS. 9–14. Photomicrographs of conidiophores and conidia of *H. pedrosoi.* × 1000.

FIG. 15. *H. pedrosoi.* × 800.

FIG. 16. *H. algeriensis.* × 1000.

FIG. 17. *H. pedrosoi.* × 1000.

GRABADOS 9–14. *Microfotografía de conidióforos y conidios del* H. pedrosoi (× 1000).

GRABADO 15. *El* H. pedrosoi. (× 800).

GRABADO 16. *El* H. algeriensis. (× 1000).

GRABADO 17. *El* H. pedrosoi. (× 1000).

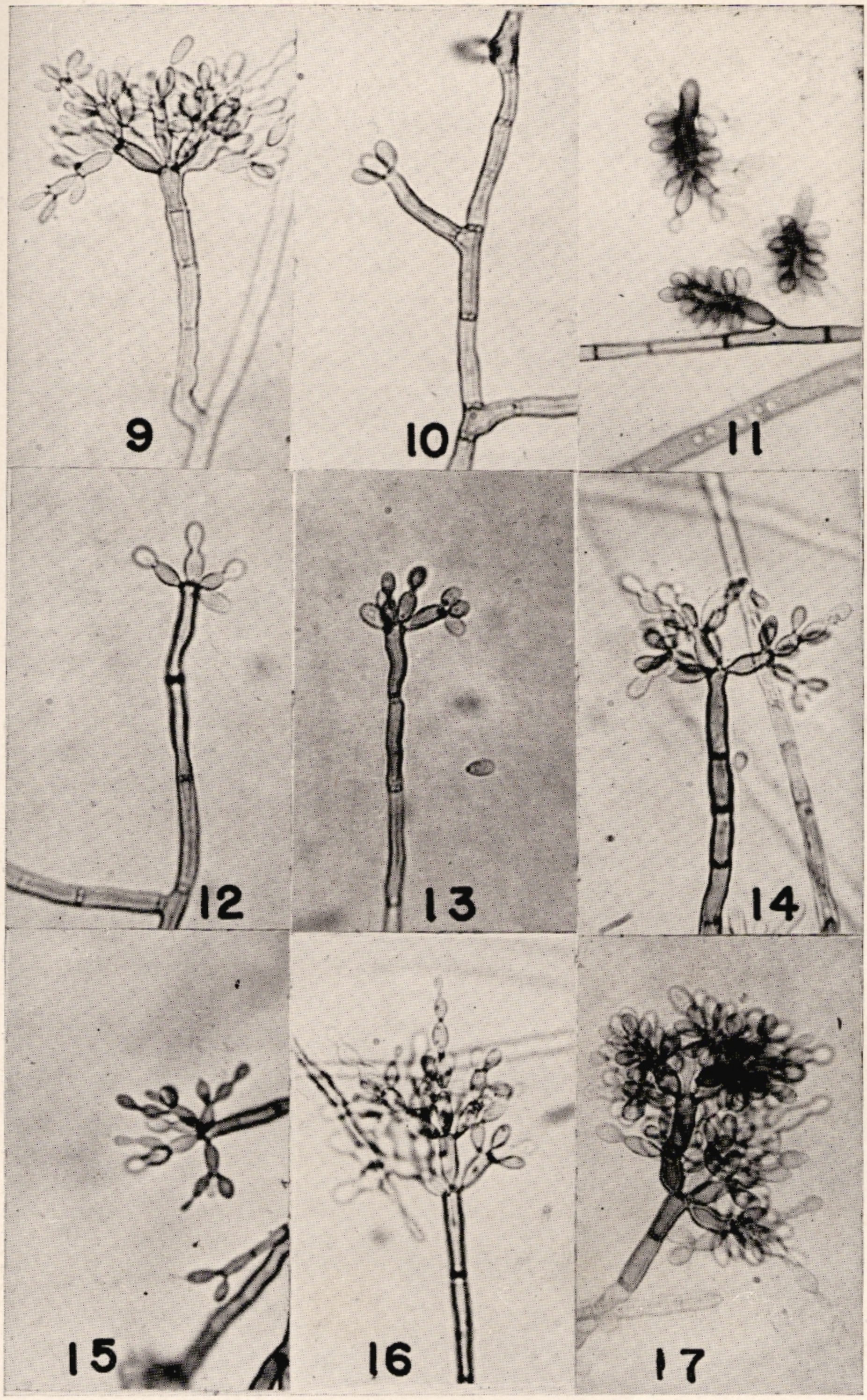

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PLATE 5

FIG. 18. Pigmented pseudoparenchymatous cells resembling those of the parasitic phase. From an old culture of *H. pedrosoi*. × 1000.

FIG. 19. "Disjunctors" on spores of *H. pedrosoi*. × 2250.

FIGS. 20–22. "Disjunctors" on spores of *H. pedrosoi*. × 1000.

FIGS. 23–24. *H. elatum*, a saprophytic species. × 1000.

FIG. 25. Branching conidial chains from an old culture of *H. pedrosoi*. × 1000.

FIG. 26. A conidiophore of *H. pedrosoi* bearing two normal spores and two conidiophores of the Phialophora type arising from modified spores. × 1000.

GRABADO 18. *Células seudoparenquimatosas pigmentadas semejantes a las que se observan en el período parasitairo. Cultivo viejo de* H. pedrosoi. (× 1000).

GRABADO 19. *"Disjuntores" en los esporos del* H. pedrosoi. (× 2250).

GRABADOS 20–22. *"Disjuntores" en los esporos del* H. pedrosoi. (× 1000).

GRABADOS 23 y 24. Hormodendrum elatum, *especie saprofítica.* (× 1000).

GRABADO 25. *Cadenas ramificadas de conidios en un cultivo viejo de* H. pedrosoi. (× 1000).

GRABADO 26. *Un conodióforo de* H. pedrosoi *con dos esporos normales y dos conidióforos de tipo Phialophora que nacen de dos esporos anormales.* (× 1000).

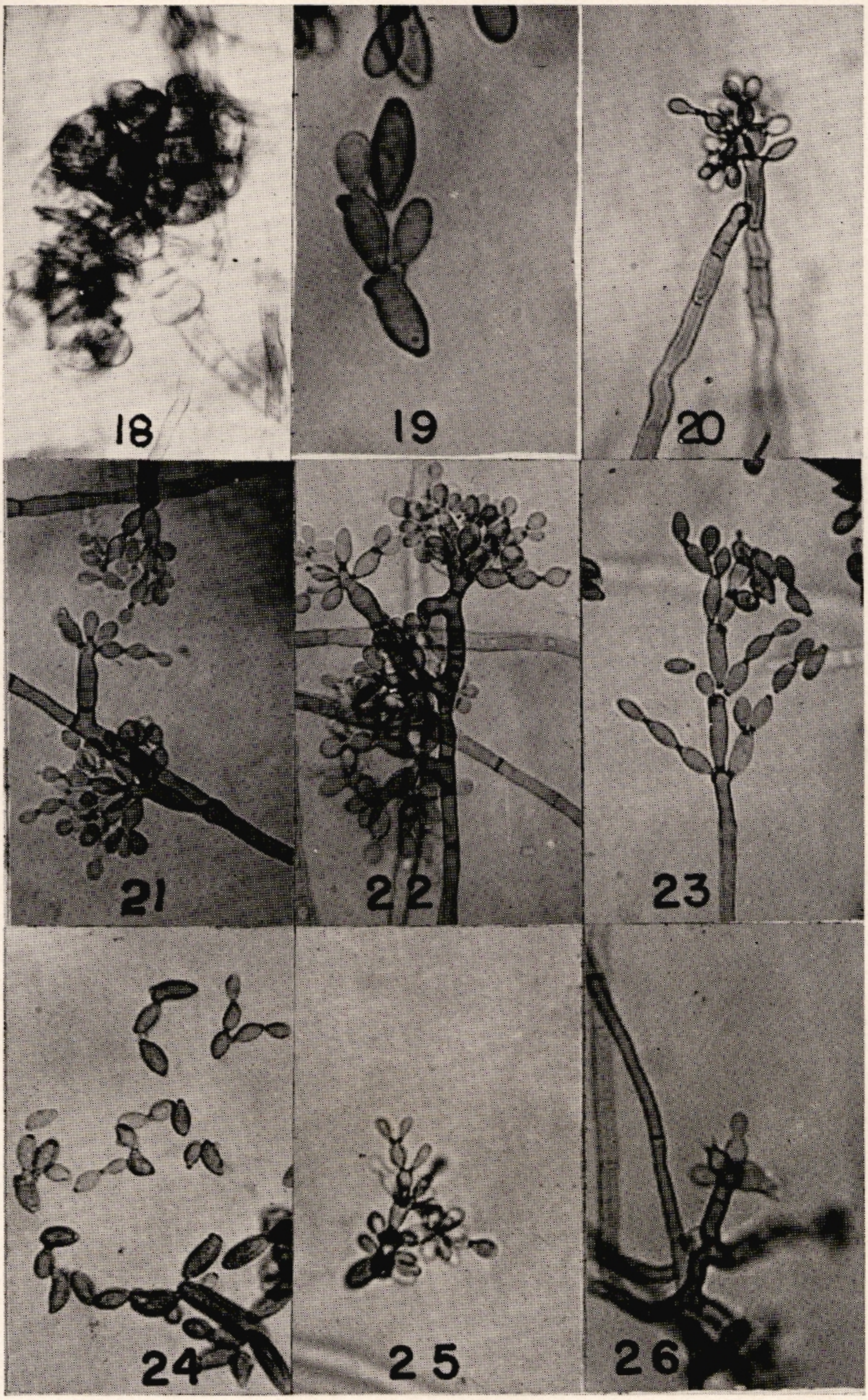

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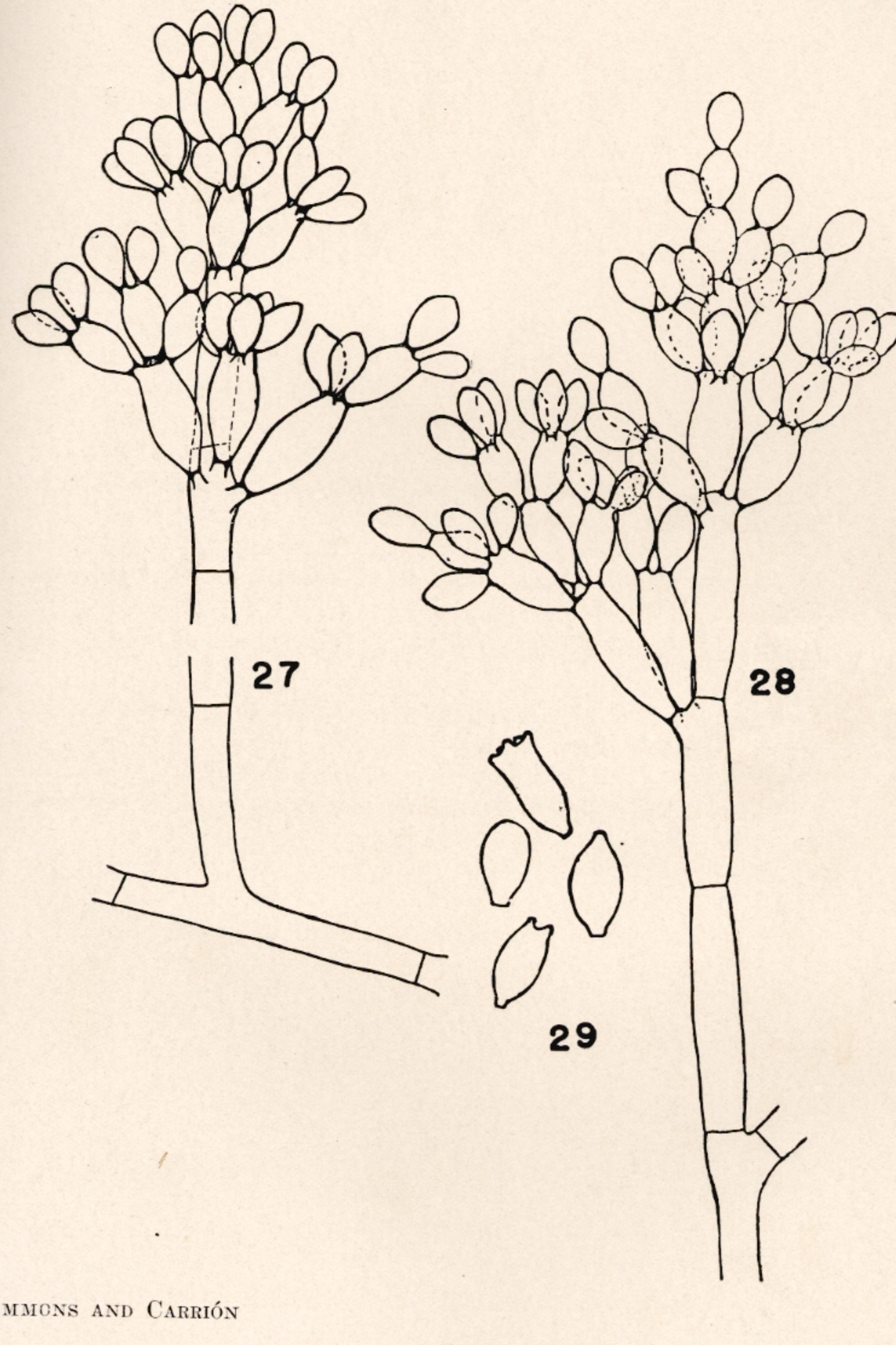
PLATE 6

FIGS. 27–28. Conidiophores and conidia of *H. pedrosoi.* Camera lucida drawings. × 2100.

FIG. 29. Modified and unmodified conidia of *H. pedrosoi.* × 2100.

GRABADOS 27 y 28. *Conidióforos y conidios de* H. pedrosoi (*Dibujo con cámara clara.* × 2100).

GRABADO 29. *Conidios de variada morfología en el* H. pedrosoi. (× 2100).



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