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CULTURED ON ARTIFICIAL MEDIA FOR 2, 3,
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VARIATIONS OF CERTAIN CULTURAL,
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OBSERVATIONS ON PATHOGENIC FUNGI CULTURED ON ARTIFICIAL MEDIA FOR 2, 3, 4, AND 5 DECADES. PERMANENCY AND VARIATIONS OF CERTAIN CULTURAL, BIOCHEMICAL AND ANTIGENIC CHARACTERS

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SUMMARIVM — Cum post annum 1920 — contra quae antea opinabantur qui harum rerum sunt studiosi — multae observatae sint variationes ac mutationes fungorum, qui quidem arte producti sint, Auctor ex sua ipsius observatione in tres classes fungos, qui hominum sint, ut aiunt, parasitae, distribuit, distinguens:

a) fungos, quorum notae nihil vel minimum mutantur, etiam post aliquot annos culturae artefactae;

b) fungos, quorum notae possunt quidem mutari, vel gravissime; sed post certum tempus, quod plerumque diuturnum est, primigenias notas de novo acquirunt;

c) fungos, quorum notae ita mutantur, ut ad pristinam speciem numquam redeant.

At the beginning of this century and up to the early « twenties » the majority of mycologists and bacteriologists believed in the 'fixity' of mycetes and bacteria: the cultural and physiological characters of these organisms would not vary significantly if grown under standard nutritional and environmental

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conditions. The only change admitted, a biological one, was a loss of pathogenicity in old strains. Then the pendulum swung the other way and a flow of publications commenced which, continuing unabated to the present day, has detailed the spontaneous changes and variations of fungi cultivated on artificial media.

The purpose of this communication is to make a few remarks on the subject, based chiefly on the subject of certain pathogenic fungi isolated long ago and kept under practically continuous investigation ever since, being subcultured on the average once a month on glucose agar (four per cent until 1930, then two per cent). The intention is merely to quote facts and to relate observations without making any attempt at an explanation of their causation.

In my experience fungi of human origin can be separated into three groups:

1. Those whose characters do not change, or only in a minimal way, through the years of subculturing on artificial media.
2. Those whose characters may change, even profoundly, but which after intervals, usually long, resume their original characteristics.
3. Those whose characters change permanently and irreversibly.

GROUP I. — FUNGI WITH PERMANENT CHARACTERS

The expression 'permanent characters' must be taken *cum grano salis*. Is there anything in nature which is absolutely changeless and immobile? Doubtless changes occur all along, but they are minimal, and for practical purposes may be ignored. As examples of this group of fungi mention may be made of certain species of *Candida*, particularly certain strains of

certain species, e.g. *Candida krusei* (CASTELLANI, 1910) BERKHOUT, 1923. The original strain isolated from sputum in Ceylon in 1909 is still extant. When first isolated it produced on glucose agar a growth which was rather dry with a finely granular or very delicately creased surface.

Microscopically the free yeast-like cells were ovoid or somewhat elongated and pseudomycelium was formed. Among the six 'sugars' which are at present generally employed for yeast identification: namely, glucose, maltose, lactose, saccharose, galactose and inulin, it produced fermentation with gas only in glucose. Among other sugars apart from the standard six it fermented only levulose and mannose. This strain has been re-investigated by a number of workers, among whom are SPAAR (1926), ZEPPONI (1931), URSO (1950), and CAPOCACCIA (1956, 1962). No change has been noted. At present, over fifty years after isolation, the strain produces a colony on glucose agar which is somewhat dry, and its surface is finely granular or very delicately crinkled. Microscopically the cells are ovoid or elongated; of the six standard sugars only glucose is fermented, and of the other sugars only levulose and mannose.

Still extant also in the author's collection are the original strains of *Candida tropicalis* (CASTELLANI, 1910), BERKHOUT, 1923, *C. pseudo-tropicalis* (CASTELLANI, 1911), BARGAL, 1931, *C. macedoniensis* (CASTELLANI, 1919), BERKHOUT, 1923. Like *C. krusei* they have remained unchanged culturally and biochemically through the years of cultivation on glucose agar. An interesting point is that *C. tropicalis*, which was found definitely virulent and pathogenic for laboratory animals on first isolation, was still so after four decades of continuous subculturing (URSO, 1950).

It would appear also that antigenically the original strains of *Candidae* isolated so long ago have undergone very little, if any, change. In 1934, at the Ross Institute in London, Dr. MACKENZIE DOUGLAS and the author carried out a sero-

logical investigation of *Candida* fungi by means of agglutination and complement fixation tests. They recognised four serological groups:

Serological Group I comprising *C. albicans* and *C. tropicalis*;

Serological Group II comprising *C. macedoniensis* and its varieties;

Serological Group III comprising *C. pseudotropicalis* and its varieties;

Serological Group IV comprising *C. krusei* and its varieties.

Groups I, II and IV were well-defined and clear-cut; Group III far less so, in fact badly defined.

A few years ago in my laboratory in Lisbon repeated investigations gave practically the same conclusions. Recently intensive serological studies on the yeasts in general, including *Candidae*, have been carried out in a number of scientific institutes in America, England, Japan and other countries, and in Germany an excellent monograph on serological mycology has been published by HANS SEELIGER.

GROUP 2. — FUNGI SHOWING REVERSIBLE CHANGES

In this group there may be mentioned as examples *Trichophyton balcaneum* CASTELLANI 1916, and *Geotrichum mata-lense* CASTELLANI 1915, the original strains of both of which still exist.

Trichophyton balcaneum — which is probably only a variety of, *Tr. glabrum* Subouraud — was isolated during the First World War, in the Balkans, from of a peculiar, diffuse, scaly condition of the scalp, resembling a severe form of *pityriasis sicca* rather than *tinea*. It grew on glucose agar, pro-

ducing a somewhat nodular or crinkled or slightly convoluted, flattened, glabrous, dirty, whitish or beige colony; it liquefied gelatine rapidly; it clotted milk. Microscopically no *macroconidia*, no spirals and no nodular or denticulated bodies were seen; only mycelial threads with a few, very doubtful *microconidia*. The fungus continued to show the cultural characters mentioned above for a number of years until 1928, when definite changes appeared: the colonies became covered with whitish duvet (aerial mycelium), not very long but quite abundant, and on scraping it off the growth appeared as a smooth, flattish mass, not nodular nor crinkley, nor convoluted in parts. It appeared to have become pleomorphic. These new features remained unchanged for about a year, and then the aerial mycelium disappeared, and the colonies began to show again a glabrous, somewhat knobby, slightly convoluted aspect. A few years later the colonies once more became fluffy, and since then these totally different appearances of the colonies have alternated at long irregular intervals of years, although the medium has always been the same, i.e. glucose agar four per cent until 1930, later two per cent. At present the cultures have the same appearance as on first isolation. The microscopical characters never varied, macroconidia, spirals, nodular and denticulated bodies and other specialised structures have always been absent.

Geotrichum matalense CASTELLANI 1915. The original strain isolated in 1914 is still in the author's possession. When first isolated and for years after the cultures on glucose agar appeared fluffy and whitish, and when this duvet was scraped off the surface of the colony was smooth. Then in 1928, after continuous subculturing on glucose agar for 13 years (approximately once a month), the duvet disappeared and the growth became deeply rugose and somewhat convoluted in parts, with a smooth, glabrous, somewhat moist-looking surface. Some years later, in 1934, the original characters with plenty of duvet came back. In 1936 the duvet again disappeared, to return

a few months later. At present the cultures show absolutely the same characters as when first isolated, with abundant whitish aerial mycelium present.

GROUP 3. — FUNGI SHOWING IRREVERSIBLE CHANGES

Every medical mycologist is well acquainted with the irreversible changes shown by certain dermatophytes when grown for long periods on artificial media, especially glucose agar. The fungus loses its original characteristics, becomes fluffy, and microscopically macroconidia, microconidia, spirals, nodular and denticulated bodies are no longer seen, only sterile mycelium being present. The original characters never return: in fact it may be said that new degenerate races, possibly species, arise with features of their own which remain permanent. This phenomenon was first studied by SABOURAUD who introduced the term 'pleomorphism', not to be confused with 'polymorphism', to indicate it. Once a fungus has become pleomorphic, it remains so indefinitely. The subject of pleomorphism has been treated thoroughly in many books, monographs, and innumerable scientific papers, especially by French authors, and fairly recently in America by REISS and LEONARD (1956). Long ago SABOURAUD (1910) proclaimed that the use of a poor medium is the best way of preventing pleomorphism, and for this purpose he devised his maintenance agar, which is prepared with peptone water instead of broth, and contains no glucose or any other sugar. None of the media recommended subsequently, including the so-called natural media and media containing various chemicals and antibiotics, have been completely successful - far from it.

In recent years a very simple procedure to minimize the development of pleomorphism has been introduced by me, which so far seems to be successful: 'cultivation', so to speak,

in plain sterile distilled water. It is based on certain experiments carried out in the Mycological Department of the London School of Hygiene and Tropical Medicine in the years 1938-39, the results of which were published with the title « The Viability of Some Pathogenic Fungi in Sterile Distilled Water » in the *Journal of Tropical Medicine and Hygiene* (London) in August, 1939.

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