CRYPTOMYCES PLEOMORPHA: A NEW ORGANISM ISOLATED FROM THE BLOOD OF A CASE OF METASTASIZED CARCINOMA OF THE BREAST*

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THIS report is presented for the following reasons. (1) The organism was detected in the circulating blood by direct examination (Fig. 1). (2) It was detected among the tumour cells in the original neoplasm, in sections which had been made four years previously (Fig. 2). (3) An organism, evidently of the same type, has been found in seven previous cases, but its cultural characteristics and pathology had not presented the same degree of completeness. (4) Though in part resembling other fungoid organisms which have been described before in human pathology, this one seems to present additional distinctive and interesting features of its own. (5) Careful observations of the living cultures have shown that in some phases this organism exactly mimics the cell-elements of human blood, and also the so-called Plimmer bodies and Russell fuchsinophile bodies of some malignant tissues, and, in addition, has forms which are just like the free nuclei and various sized granules found in many sections of neoplasms. These mimicries explain how the organism may effectually escape detection in the routine observation of tissues and blood.

Isolation.—From the blood of a case of carcinoma of the breast (excised five years ago) with intrathoracic metastasis; previously, in other cases, from the tumour tissue.

Cultural characteristics.—The initial growth appeared only after seven days' incubation at room temperature. After developing a raised formation like that of sporotrichum, but extremely hard in consistence, it remained stationary for two weeks, despite repeated attempts at subculture on a great variety of media of all gradations of pH. Finally, the use of the patient's serum, and asparagus extract to enrich the glycerine-glucose-peptone water, proved to induce cultural success, good growths now appearing in all subsequent cultures in 48 hours. The organism was strictly aerobic. Room temperature was best. The optimum pH was 6.8 to 7.0. Bacterial contamination effectually antagonized the cultures.

On *asparagus agar.*—Within 48 hours a small round orange-tinted colony appears with elevated glistening centre. As this gradually enlarges it becomes coral pink. A somewhat hardened pellicle forms in the centre and delicate threads form at the periphery, at first whitish, and, later, faintly coral-pink (Fig. 1). Only spore forms occur in the initial colony. Hyphæ develop in from 6 to 10 days, showing various forms as the culture ages (Figs. 17-31). After 14 days the pink centre wrinkles up, much like sporotrichum, *Mycobact. tuberculosis,* etc., and the whole growth constitutes quite a firm membrane rather firmly adherent to the

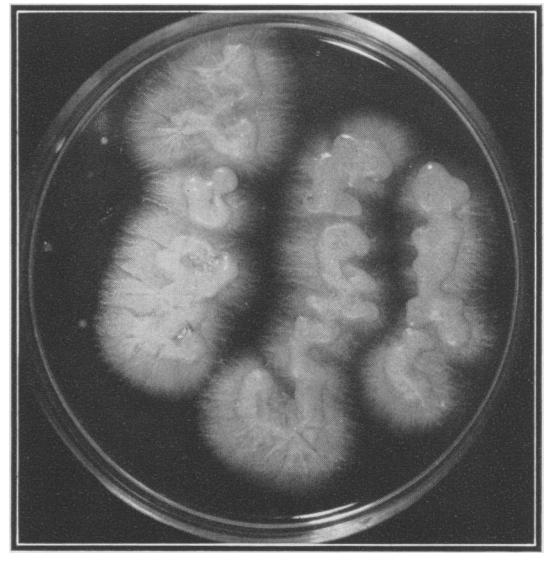

FIG. 1.—Petri dish culture, 12 days old. The central raised growth consists of the torula-like spore forms, and is of coral colour in the original. The delicate hairy outgrowth is the mycelial phase.

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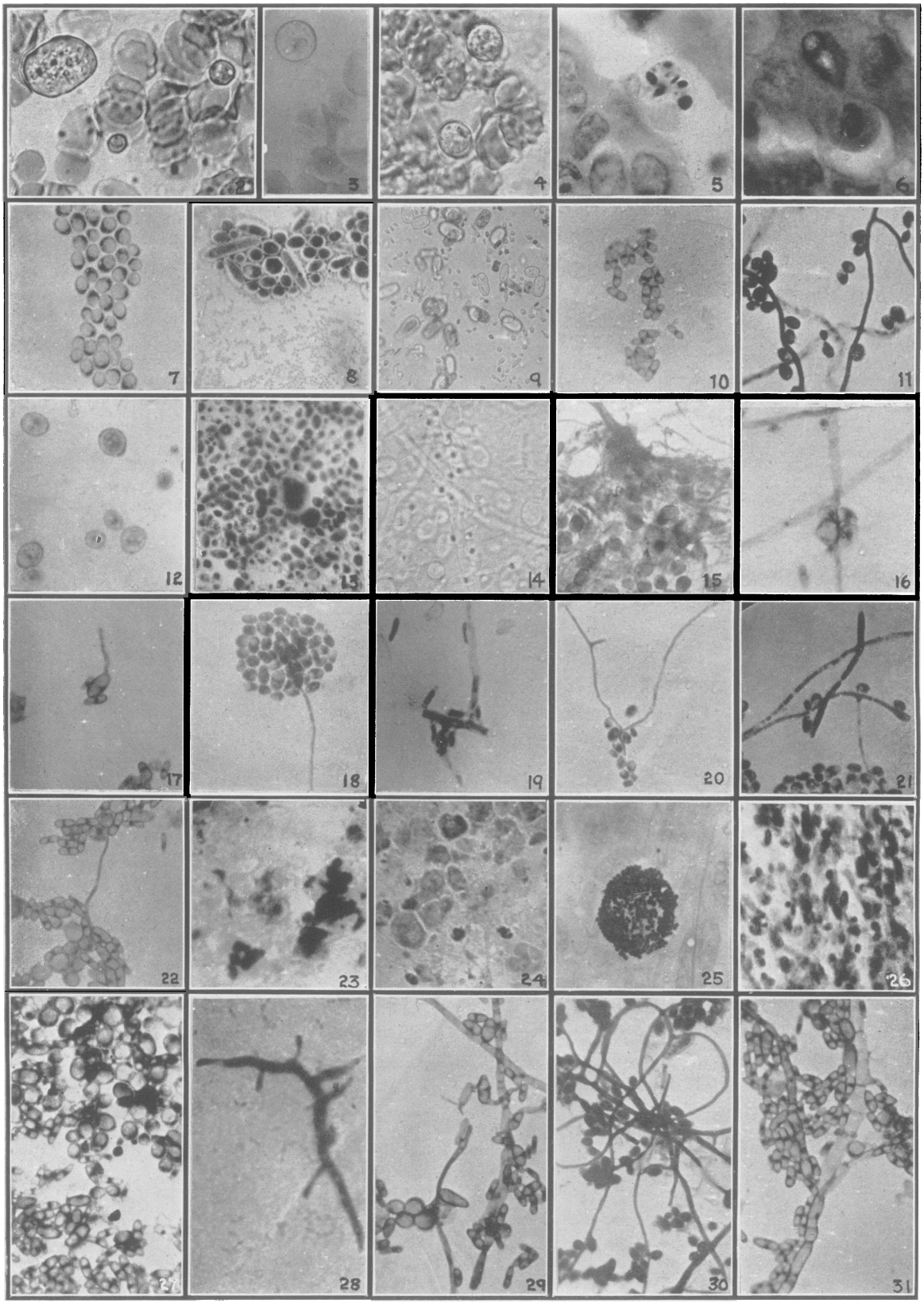
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EXPLANATION OF FIGURES

FIGS. 2, 3, 4.—From three fields showing the spherical bodies in the fresh unstained blood. Note differently sized bodies, the larger ones being the same size as the red cells, forming flattened discs. Micro-spores (very refractile bodies) are seen among the cells; these are the same size as platelets. (Oil-immersion lens).

FIGS. 5, 6.—Two fields from the original tumour tissue, showing similar forms free in the lymphatic spaces. (Oil-immersion lens).

Figures showing pleomorphism in the same culture at different dates. (Magnification in Figs. 7 onwards, Oc. 15, Zeiss, and obj. 20).

FIG. 7.—Rounded forms like blastomyces (4 days).

FIG. 8.—Elongation of these rounded bodies into tubular forms, with halo or capsule.

FIG. 9.—Oval forms like those found in "blastolysin".

FIG. 10.—Fission-forms, or oidia (10 days).

FIG. 11.—Budding forms like yeasts (5 days).

FIG. 12.—Megaspores or ascospores with highly refractile capsule, like those in Figs. 2–4.

FIG. 13.—Torula forms in thick smear, mimicking tumour-cell nuclei

FIG. 14.—Highly refractile micro-spores, unstained; there is a background of torula forms and two hyphæ.

FIG. 15.—Chlamydospores among mycelium (14 days).

FIG. 16.—Cluster of megaspores appearing as a sessile sporangium. Stages of mycelial growth.

FIG. 17.—Germination of diplo-spore (36 hours).

FIG. 18.—Cluster of spores only one of which germinates.

FIGS. 19, 20, and 21.—Secondary branching (3 days).

FIGS. 22 and 29.—Appearance of forms like those in Fig. 7 (10 days).

FIG. 23.—Smear from nodule in rat which had been inoculated with the organism in the submammary region. Note the megaspore forms with clear capsule.

FIG. 24.—Similar smear from another rat similarly treated; note the oval darkly stained bodies.

FIG. 25.—Splenic smear from rat dying of generalized septicæmic infection, showing morula mass of budding toruloid forms (Leishman stain).

FIG. 26.—Section of nodule at site of inoculation, showing spindle cells with yeast-like forms intermingled.

FIG. 27.—Transitions from oidia form to blastomyces form (tenth day).

FIG. 28.—Early mycelium with lateral buds like sporotrichum (fifth day). (Oil-immersion lens).

FIG. 30.—Loop-formation and the beginning of a complex hyphal mass, with rapid formation and multiplication of buds, which themselves bud again.

FIG. 31.—A thick hypha in which oval torulæ seem to be forming; splitting into oidia is commencing (14 days). In these last two figures the existence of + and — forms is suggested.

surface of the medium. Finally, a very fine white powdery deposit appears all over the growth, and no further change occurs.

On *asparagus glucose glycerine peptone water,* a pellicle forms in 48 hours; this is in the form of a ring at the surface, adhering to the glass, and made up almost entirely of mycelia, the spores falling to the bottom of the tube in large numbers. A pink colour appears in 7 to 10 days, and the ring can be detached from the glass, when it will remain intact and hanging in the medium.

Gelatine.—Not liquefied; very slow growth. *Meat-extract media.*—Very slow growth; colonies very small; whitish; no colour. *Plain glucose glycerine agar.*—Extremely slow growth; small glistening colonies; no colour; no mycelium. *Sabouraud.*—Very slow colourless growth; very small and few hyphæ; spores much smaller than those on asparagus media. *Dextrose-tartaric acid.*—No growth (in distinction from usual fungi). *Potato.*—Very scanty dull yellow growth, slowly turning pinkish. *Tomato.*—Very luxuriant growth in 48 hours; pink colour slightly paler than the flesh of the fruit. The condensation water rapidly fills with torula forms and turns pink. *Milk.*—Budding torula forms develop in 72 hours; no coagulation; no acid. *Peptone water.*—No indol. *Nitrate media.*—No nitrite formation. *Sugar-reactions.*—No fermentation of glucose; gas, but no acid in lactose; saccharose, nil.

Stainability.—Dilute carbol-fuchsin gives the best results. Methylene blue stains relatively feebly. It is Gram-positive. It is not acid-fast. Rossophilia is almost absent.

Morphology. — This organism is very pleomorphic. Thus at different times, in the same culture tube, one may see transient micrococci like Doyen's[1] M. neoformans of 1904 (Fig. 8), spherical forms like Sanfelice's blastomyces (1896) (Fig. 7), tubular forms apparently answering v. Brehmer's[2] description of his Siphonospora (Fig. 8); oval resting spores like those found in a specimen of Schmidt's[3] "Blastolysin" (Fig. 9); combined with mycelial formations (Fig. 11).

The following forms of reproduction are observable, as met with in other fungi: (a) fission forms or oidia, or diplospores (Fig. 10) suggestive of schizosaccharomyces; (b) bud-formation as usual in torula or saccharomyces (Fig. 11); (c) ascospores, or "megaspores" (Fig. 12) in keeping with zygosaccharomyces; (d) very minute microspores (Fig. 14), which are highly refractile and arise by detachment

from (b); these are metachromatic with Leishman and with methylene blue; (e) chlamydospore formation (Fig. 15); (f) lateral buds (Fig. 28), as in typical sporotrichum; (g) sessile sporangia in the aerial mat (Fig. 16), suggestive of some mucors; (h) still smaller particles occur, possibly filter-passing. There is a fairly regular sequence. Thus from b to d, from b to mycelium (first week), from mycelium to f, and to a, and to e (second week); from a to c and to g. The whole series ends in about three weeks, when a new subculture becomes essential.

The organism is therefore an ascomycete, having affinities with sporotrichum, cryptococcus and blastomyces. It is given the name of *Cryptomyces* because it combines mycelial formation with an obscure biology and incidentally an effective concealment when situated in invaded tissues.

Pathogenicity. — It is pathogenic for white rats when given intrapleurally, and for white mice when given into the submammary tissue. It was recovered true to its original cultural characteristics in pure form from the blood and all organs of these animals but developed very little mycelium. A high monocytosis was striking and early. The progeny of a female rat inoculated with the organism while pregnant developed generalized infection with the same organism, dying 21 days after birth. Subcutaneous inoculation produced indurated areas in which the organisms were plentiful. After 12 days nodules formed in which spindle-shaped connective-tissue cells were predominant, and megaspores were abundant among them (Fig. 26). Mycelium was not detected in the tissues.

Cuti-reaction.—Skin reactions have been obtained by using an emulsion of this organism on the patient whence it was derived, as well as on several other malignant cases, including sarcomas, but not on persons in ordinary health. Further studies on this point are in progress.

Relation to malignant disease. — Naturally, such an organism may be primary or secondary. If the former it would presumably act in virtue of some carcinogenic substance being formed among the products of its metabolism. In any case, the frequency of its association with new-growths remains to be established. The usual arguments against a microbic origin of the disease naturally arise.

DISCUSSION

An accidental admixture of two or more organisms would provide a simple explanation of the pleomorphism. The two main forms have already been separately described from time to time as being causal for cancer, to be subsequently rejected as "contaminants", namely, the yeast-like forms and the mucor-like forms. Moreover, in the present case, the cultures after passage through the infected rats and mice showed a dominantly blastomycetic form, with mycelial formation almost negligible. Further, the patient had been for a few weeks in a region where sporotrichosis occurs, though never having the slightest evidence of lesions of that kind.

The reasons for believing the organism to be single are: (a) the constancy of the occurrence of the dual forms in succession, at the same time intervals in all subcultures; (b) the ability actually to observe the one form changing into the other; (c) the inability to separate them permanently by plating; (d) the cultural characters; (e) the possession of pathogenicity; (f) its unique character; (g) the unlikelihood of a chance admixture of distinct organisms resulting in such a close symbiosis as actually to manifest conjugation.

Of some interest is the experience of the animal lesions produced, in that the organism is found closely mingled with the reactive infiltration of predominantly monocytic type, and shows in the sections appearances which strongly recall those seen in sections of carcinomas and sarcomas, though such particles are usually regarded as unessential bacterial or degenerative components. Applied to human tumour histology, the presence of such microscopic objects would seem to require an explanation.

Of further interest is the evidence of sexuality in this organism (in common with other fungi), though the question of haploid and diploid phases has not been taken up. The delay in obtaining good subcultures would be explained by the difficulty of finding a medium favourable for the conjugation of the + and − elements. This provides a useful suggestion—that such occupants of tumour tissue (whatever their presence means) present a sexuality factor which determines ease or difficulty of culture outside the body. The repeated observation of the megaspore forms inside the phagocytic cells in the infected animals with their mimicry of the Plimmer-body and other

inclusions in human cancers supports this suggestion quite definitely.

CONCLUSIONS

A report is given of a new organism, here called *Cryptomyces pleomorpha,* which is placed among the ascomycetes.

The question of etiological relationship with malignant disease is necessarily left open.

Very grateful thanks are due to Dr. Archibald and to Dr. Mark Kaufmann for enabling this case to be worked out, and also to Dr. Pauline Beregoff, for recently undertaking further tests on animals.

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THE REACTIONS ATTENDING THE INTRAVENOUS USE OF THE ARSPHENAMINES*

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THE physician who would explore the therapeutic possibilities of the arsphenamines should be thoroughly cognizant of the various reactions which occasionally follow their use. The causes of the reactions are probably as diverse as are the reactions themselves, and, though not yet fully understood are gradually becoming clearer as a result of intensive study by numerous investigators.

Arsphenamine was originally prepared by Ehrlich and Bertheim by the reduction of 3–amino 4– hydroxyphenyl arsenious oxide, for brevity termed "arsenoxide". This substance is twenty times more toxic than arsphenamine, and is in part reformed by exposing arsphenamine to the atmosphere. Since the work of Voegtlin and Smith,[1] later confirmed by Schamberg, Kolmer and Raiziss,[2] showing that arsenoxide was many times more trypanocidal than arsphenamine or neo-arsphenamine *in vitro,* there has been a growing conviction that arsenoxide formed in the body by the oxidation of arsphenamine is responsible for the therapeutic effect and also is partly the cause of its toxicity. Schamberg, Kolmer and Brown[3] have recently shown that the presence of arsenoxide in solutions of arsphenamine and neo-arsphenamine shortens the latent period for a trypanocidal effect when injected into rats with trypanosomiasis, but does not change the minimal curative dose per kilo of weight as compared with solutions free of arsenoxide. In other words, arsenoxide is rapidly trypanocidal, whereas arsphenamine is more slowly so, the time difference being required to oxidize the arsphenamine to arsenoxide in the tissues. The toxicity of the arsphenamines, in so far as arsenoxide is concerned, probably depends upon variation in the rate of oxidation in different individuals.

Arsenoxide is by no means the sole factor in toxicity. It is well known that acid solutions of arsphenamine produce a precipitate in the blood and may cause pulmonary embolism, and Milian[4] has shown that similar effects may be due to a relatively low alkalinity of the blood of the patient. It was formerly thought that neo-arsphenamine and sulpharsphenamine did not cause precipitates, but it has been shown by Shivers[5] that acid solutions of these drugs are quite capable of precipitating the plasma proteins. Jean Oliver and associates[6] proved that large doses of arsphenamine caused an agglutination of red cells, but only in the presence of an electrolyte such as sodium chloride. This agglutination and the subsequent vascular injury resulting from the thrombi can be prevented by the use of a buffer such as gelatin. Reactions may also be caused by impurities in the diluent and, as pointed out by Stokes and Busman,[7] sometimes rubber tubing contains, when new, a toxic agent which may be taken up by the solutions, causing them to produce febrile reactions when introduced into the blood stream.

THE NITRITOID CRISIS

The most frequent reaction is the nitritoid crisis, so named because of its similarity to the

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