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MENINGO-ENCEPHALITIS CAUSED BY CRYPTOCOCCUS NEOFORMANS

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While some years ago the opinion had prevailed that certain diseases caused by fungi occur only in certain parts of the world, it has lately become more and more evident that fungal diseases are not restricted to geographical areas.

Infection with Cryptococcus neoformans had not been reported from Hungary before 1954 when we had occasion to observe a patient[20] and, simultaneously also another case was reported[16]. Clinical course and pathological findings were characteristic in our case and the histological examination had allowed to make some remarkable observations.

The patient, a 76 years old female, was admitted to the 1st Medical Department of our hospital. According to her relatives, about two months before she had caught a cold. Her physician had diagnosed pneumonia and pyelitis. For three weeks she had had a cough, and a temperature of about 39° C (102,2° F). Then, on the effect of penicillin, antipyretics and codein the condition had gradually improved, temperature had become normal and the cough had subsided. Subsequently she had complained of lasting headaches now and again accompanied by vertigo, nausea and vomiting. Her gait had become uncertain, with numbness of the legs. She had been extremely restless, could not sleep and had lost 15 kg in weight in 8 weeks.

On admission, the pulse frequency was 60/min ; blood pressure 180/90 mm mercury ; temperature was normal. Respiration was scarcely audible, the pupils showed hardly any reaction ; the patellary reflex could not be elicited on either side ; Babinski's sign was positive on both sides. She died the morning after the day of admission. Clinical diagnosis was hypertension, encephalomalacia.

Post mortem the following were revealed. The body surface was intact. In the superior lobe of the left lung there was a circumscribed, greyish-white, mucous node of the size of a nut, of liver consistency, homogeneous on the cut surface. The thoracic and abdominal organs were normal. The calvaria was medium thick, yellowish-white, the dura medium taut, the pia mater oedematous. In the basic grey nuclei numerous, symmetrically arranged cysts of the size of a pin-prick, a millet seed, a lentil were found, filled with a white slimy substance (Fig. 1).

In the sections prepared from the lung and the brain numerous round or slightly oval fungi, measuring 5—20 μ for the most part sorrounded by a broad capsule csould be observed (Fig. 2). In the lung the groups of fungi were separated by connective tissue septa, while in the brain they were arranged in smaller or larger cysts, whose walls were not lined by cells and no inflammation was seen around them. In the subarachnoidal space there were fungi of different size, arranged in several layers ; at places in the cortex immediately beneath the pia mater the development of cysts was observed. In the brain substance perivascular lymphocytic infiltration was found at one place only, while on the meninges there were, apart from the fungi, only single inflammatory foci consisting of some lymphocytes.

The post mortem diagnosis was cryptococcic meningoencephalitis, toruloma in the left lung.

The fungus had not been cultured to support the diagnosis. The classical histological pattern found has, however, proved without any doubt that the condition was due to the fungus officially termed today Cryptococcus neoformans. Before it had been termed Torula histolytica and the disease caused by it was often called blastomycosis. On the strength of recent knowledge, infections termed by the latter name may be divided into three groups, viz.(i) North American blastomycosis, caused by Blastomyces dermatitidis (GILCHRIST),

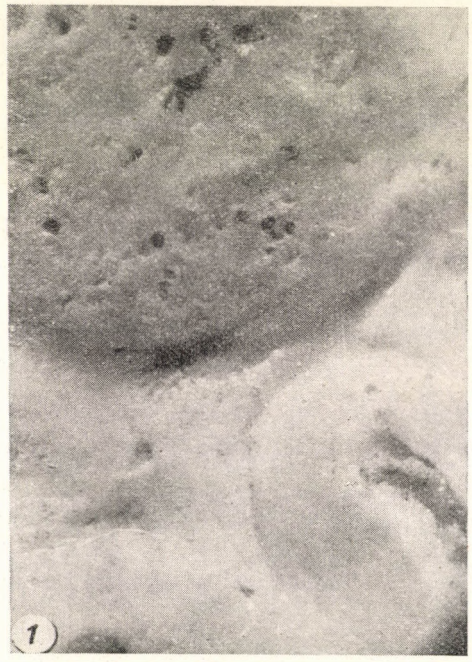


Fig. 1. Numerous cysts of the size of a pin-prick or millet seed are visible in the grey substance of the basal nuclei (×2)

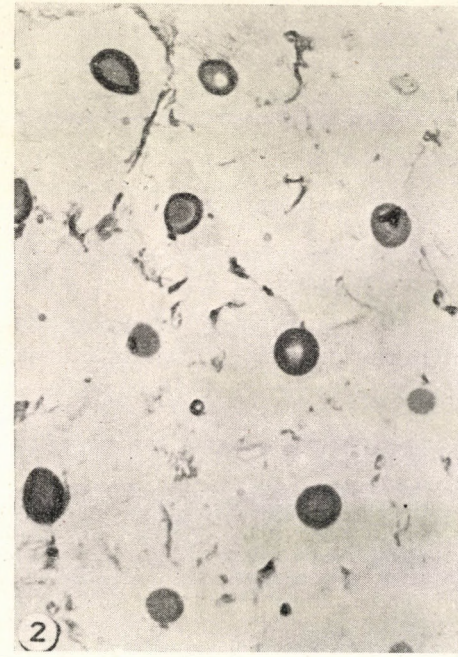


Fig. 2. The cryptococcus neoformans is a round or slightly oval organism, measuring 5μ by 20 μ. Azan stain (×400)

(ii) South-American blastomycosis or paracoccidioidomycosis caused by Blastomyces brasiliensis (LUTZ—SPLENDORE) and (iii) the disease due to Cryptococcus neoformans (BUSSE—BUSCHKE, SAN FELICE—VUILLEMIN), called formerly European blastomycosis or torulosis. Considering that, as found in tissues, from among the above agents only Cryptococcus has a broad mucous capsule, our findings have confirmed the diagnosis also without culturing.

In connection with our case the problem arose from where and by what route infection had been contracted. According to the literature[14], the fungus was recovered as a saprophyte from insects, bees, in wine-and beer-cellars, from food and fruit juices, sometimes from the human skin, the nasal mucous mem-

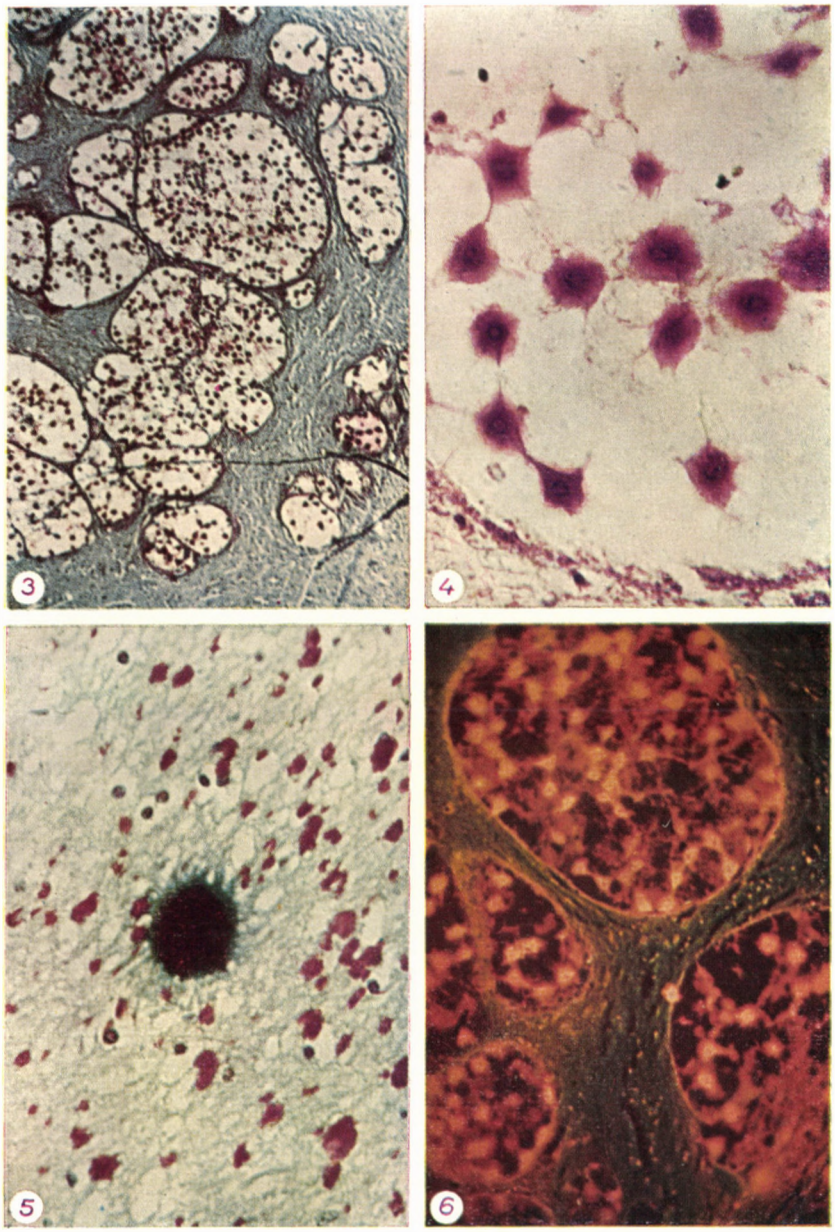
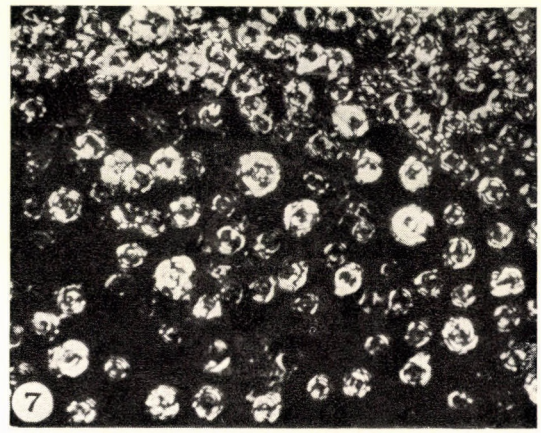


Fig. 3. Cysts situated in the grey substance contain fungi showing metachromaisa. Toluidine blue (×20)

Fig. 4. Mucous capsule around the fungi shows a medium intensive periodic acid-Schiff positivity. Periodic acid-Schiff reaction (×400)

Fig. 5. On the periodic acid-Schiff reaction combined whith the Hale method the cryptococci stain blue; corpora-amylacea like decomposition products stain purple. Ritter—Oleson reaction (×400)

Fig. 6. Under the fluorescence microscope the red colour of the fungi is in sharp contrast with the blue blase. Acridine-orange stain (×80)



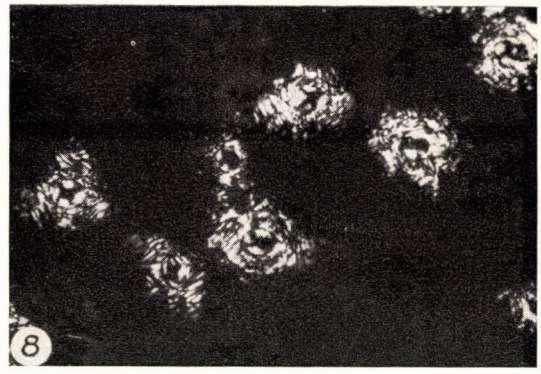


Fig. 7. Under the polarizing microscope the fungi appear as polarizing crosses. Acridine orange stain (×80)

Fig. 8. Under high power magnification the doubly refracting capsule developed around the polarizing crosses is also well visible. Acridine-orange stain (×400)

brane, the digestive tract. Some investigators could even observe it to turn into a pathogenic organism. Thus, in our case, there had existed many possible sources of infection. The fungus introduced into the human organism induces most frequently lesions in the respiratory tract, disorders of the nervous system. Recently it has also been observed in connection with lesions of the lymphoid apparatus[15]. The incubation period of the disease is unknown. Infection of the respiratory tract appears with symptoms of a common cold, a rise in temperature ; later on also pneumonia may develop. The X-ray finding is not characteristic. The involvement of the nervous system, accompanied by headache, nuchal rigidity, vomiting, sleeplessness, a rise in temperature and occasionally by focal symptoms, may be confused with tuberculous meningitis. If, on grounds of careful observation, the possibility of a fungal infection arises, the diagnosis can be established in the living by examining the CSF and isolating the organism. This is interesting with respect to prognosis but of no decisive importance there being no efficacious therapy known. Prognosis is therefore very unfavourable. This might have been one of the chief reasons which in the last ten years have prompted numerous investigators to study the problem. From among the great number of studies on the subject, the most valuable are those which, in addition to valuable data, offer an exhaustive review of the pertaining literature. Of these, the monograph of Cox and Tolhurst[7] from Australia, the parts dealing with pathology of which had been written by R. A. Willis, is the most important. By carefully observing 13 cases they had opportunity thoroughly to examine the different changes caused by the fungus. From among French authors, Debré et al.[8], as well as Segretain et al.[9, 30, 31] have published several reports dealing mainly with experimental infections. From Germany, Hoffmeister[15] in a review and Mohr[19] in a special chapter have contributed to the knowledge of the disease. The report on 165 cases by Gendel, Ende and Norman [11], published some years ago in the United States has greatly stimulated the interest in this question, as confirmed by the studies of Zimmermann[35] Moss et al. [25], Baker and Hangen [1] and of Caldwell and Raphael [4] in England.

On the strength of the data in the literature, the following motives may be advanced for the increasing interest in medical mycology.

(i) The modification of the microbial flora caused by antibiotics. (ii) The favourable response to therapy of certain fungous infections previously believed to be fatal. (iii) The fatal outcome of fungal infections complicating other diseases, and, finally, (iv) the improvement of the demonstration of fungal infections by means of culturing, serology, skin tests, animal inoculations and the advances in histological technique.

Establishing the correct diagnosis has considerably been assisted by the introduction of histochemical methods. For demonstrating substances of a polysaccharide character, McManus [18] in 1946, Hotchkiss [17] in 1948 had

recommended a simple method, the periodic acid-leucofuchsin or the periodic acid-Schiff reaction. KLIGMAN and MESCON [17b] in 1950 have found the method suitable also for histological identification of fungi and now it is being extensively used, especially since the modification of GRIDLEY [12] and the combination suggested by CAWLEY et al. [5, 28]. The examinations of STARR et al. [32] also support the fact that the method gives great assistance in diagnosing various granulomatous diseases of uncertain aetiology [6]. The authors mentioned contend, however, that more effective and dependable methods are needed in this field. In 1954, at the Congress of German pathologists, BRASS [2] described the method of polarizing optics worked out by POTENZA et al. [3, 27].

We have carefully studied the recent methods in our own case as well as in animal experiments. The method of McManus — even in its original form — we found considerably superior to the usual staining techniques (Fig. 4). Its only drawback was that in the brain sections the round cryptococci were occasionally confounded with corpora amylacea or with other lipid-protein decomposition products. Recently, SEGRETAIN [30] has recommended the iodine reaction for differentiating the fungus. On the basis of our praevious examinations, according to which the corpora amylacea are of a lipid-protein character [21, 22], we applied a combination of the Hale method with the periodic acid-Schiff reaction, as suggested by CAWLEY [5]. By means of this technique, the corpora amylacea of a lipid character stained crimson with leuco-fuchsine, while the fungi of a polysaccharide character, adsorbing colloidal iron, stained blue with Prussian blue reaction [13] (Fig. 5).

Subsequently, although at that time we were not yet acquainted with the studies of POTENZA et al. [27] — we used the polarizing optical technique for examining the cryptococcus' thick capsule with a double contour. The result, which shall be reported elsewhere, was that certain dyes are deposited in a regular pattern among the particles of the capsule consisting of submicroscopically arranged micellae, and increase thereby the existing but feeble anisotropy to such an extent, that even mounting in Canada-balsam was not capable of paralyzing it. This phenomenon has been known since long by the biologists who call it dichroism. Its use for histological purposes has, however, been attempted by a few investigators only.

From among the many dyes tested to bring about the phenomenon, acridine-orange, one of the fluorescent dyes, has proved to be the most adequate. Subsequent to staining for 10 minutes with its 1 : 1000 aqueous solution, dehydration and mounting in Canada-balsam, the fungus could be identified by two different microscopic methods. In fluorescent light the fungi appeared fluorescing in red, in sharp contrast to the blue brain substance (Fig. 6). The same preparation examined with the polarizing optical method with crossed nicols showed the fungi golden-yellow in the dark visual fields, so that their identification took only a few moments (Figs. 7. and 8). The method also allowed rapidly to diffe-

rentiate the fungi from the above mentioned corpora amylacea, since the latter structures did not show the phenomena described.

According to our examinations of smears made from colonies, and of fresh unfixed preparations of the brain of mice experimentally infected with cryptococcus and of frozen and paraffin embedded sections of tissues fixed in formaldehyde, the cryptococcus takes the dichroitic stain only if it has been isolated from man or animals. It was established that with proper discernment the method is well suited for rapid histological identification of the cryptococcus. Only doubly refracting, periodic acid-Schiff positive substances found after deparaffinization, removal of the formaldehyde pigment and taking into account and excluding all anisotropic impurites, were regarded as fungi. We found that the two methods complete each other well. After dichroitic staining the organisms to be found in the mildly periodic acid-Schiff positive necrotic foci can be more quickly and more infallibly ascertained with the polarizing optical method, whereas the fungi with some of them located intracellularly in the epitheloid granulation tissue, probably because their capsular substance has been decomposed, do not show dichroism and become identifiable only through their medium intensive periodic acid-Schiff positivity.

In our examinations the usual staining techniques have also been applied. The cryptococcus stained blue with haematoxylin, steel blue with Azan, metachromatic with toluidine blue, brown with Bismarck-brown, Gram positive, violet-rose with mucicarmine. Its capsule gave a positive periodic acid-Schiff reaction, and stained with Bismarck brown and mucicarmine. It turned black on silver impregnation. With Mallory's dye the fungus stained blue but no bluish reaction could be observed after treatment with iodine, and Sudan III stain was not taken even in frozen sections. Treatment of both frozen and embedded sections with lipid solvents proved that lipids play no part in the structure of the fungus' capsule. The mucous halo around the capsule did not disappear on digestion with pepsin, trypsin, hyaluronidase. On the strength of the findings, as well as of the data in the literature [10] it may be assumed that polysaccharides, possibly chitin, consisting of acetyl glucosamine molecule chains, may have a part in the formation of the capsule. Our observation that the capsule disappears on hydrolysis with 1 per cent periodic acid for 96 hours, appears to lend support to that supposition. On the other hand, parallel with the disappearance of the capsule, during hydrolysis double refraction and dichroism decrease in intensity and ultimately cease completely. Organic and inorganic acids, bases, as well as oxidizing agents used as control remained ineffective. Since there are data referring to the fact that on prolonged treatment with periodic acid mucoid groups become decomposed [24, 33], a similar phenomenon might be assumed to have occurred in the present case, too. Our investigations into the structure of the capsule will be dealt with in detail elsewhere [23].

Summary

(i) A case of meningo-encephalitis following infection of the respiratory tract by Cryptococcus neoformans has been reported.

(ii) One of the causes of the increasing interest in fungal infections all over the world is the improvement of diagnostic methods.

(iii) Histologic demonstration of the fungi is considerably facilitated by the periodic acid-Schiff reaction.

(iv) A new method has been recommended for identifying the fungus. The method has two advantages ; i. e. subsequent to staining with an 1 : 1000 aqueous solution of acridine orange the fungi appear golden yellow under the polarizing microscope, while they are of a red colour in fluorescent light.

(v) On prolonged treatment with periodic acid the capsule of the cryptococcus disappears, its double refraction ceases. According to the results of treatment with enzymes, acids, alkalis, lipid solvents and oxidizing agents, mucopolysaccharides and chitin seem to have a part in the formation of the capsule.

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МЕНИНГО-ЭНЦЕФАЛИТ, ВЫЗВАННЫЙ CRYPTOCOCCUS NEOFORMANS

Я. МОЛЬНАР

1. Изложение случая менинго-энцефалита, после инфекции дыхательных путей, вызванной cryptococcus neoformans.

2. Одной из причин проявляющегося во всем мире интереса к грибковым инфекциям является улучшение возможностей диагностики.

3. С 1950 г. применение периодно-кислотно-лейкофуксиновой реакции в значительной степени способствовало патогистологическому выявлению грибов.

4. Автор предлагает новую методику для выявления криптококков, которая имеет двойное преимущество. После окрашивания водяным раствором 1 : 1000 акридин-оранжевого красителя при исследовании в поляризационном микроскопе грибы сверкают в золотисто-желтом цвете ; в флуоресцентном свете же они флуоресцируют в красном цвете.

5. На продолжительное действие периодной кислоты исчезает капсуля криптококков, их двойное преломление прекращается. На основе обработки энзимами, кислотами, щелочами, растворителями липоидов, как и окислителями можно предполагать, что в создании капсулы участвуют мукополисахариды и хитин.

MENINGO-ENCEPHALITIS, HERVORGERUFEN DURCH CRYPTOCOCCUS NEOFORMANS

J. MOLNÁR

1. Besprechung eines Falles von Meningo-encephalitis als Folge einer Infektion der Atmungswege, hervorgerufen durch Cryptococcu neoformans.

2. Das gesteigerte Interesse in der ganzen Welt an den Pilzinfektionen findet seine Begründung zum Teil in den besseren diagnostischen Möglichkeiten.

3. Den patho-histologischen Nachweis der Pilze hat die seit 1950 angewandte Periodsäure-Leukofuchsin-Reaktion wesentlich erleichtert.

4. Der Autor empfiehlt eine neue Methode für den Nachweis des Cryptococcus, die einen doppelten Vorteil hat. Nach Färben mit in Wasser gelöstem Akridin-Orange, in einer Verdünnung von 1 : 1000, leuchten nämlich die Pilze bei der Untersuchung mit dem Polarisations-Mikroskop in goldgelber Farbe auf, während sie in fluoreszierendem Licht rot fluoreszieren.

5. Bei fortgesetzter Einwirkung von Perjodsäure verschwindet die Kapsel des Cryptococcus, die Doppelbrechung hört auf. Durch Behandlung mit Enzymen, Säuren, Laugen, lipoidlösenden und Oxydationsmitteln wurde festgestellt, dass in der Ausbildung der Kapsel wahrscheinlich Mucopolysaccharide und Chitin teilnehmen.

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