TABLE I.
Toxicity.

Compound	LD_{50}	Aniline %	Aniline g
Monoanilide of aconitic acid	4.0	37	1.5
Acetanilide	2.42	68	1.6

by the method of Hardy, *et al.*,[3] as adapted for use with animals. The data in Table II show that these 2 compounds in the doses given were essentially of equal efficiency in raising the pain threshold of dogs.

TABLE II.
Analgesic Action

	Rise in pain threshold			
	Monoanilide		Acetanilide	
Dog No.	Peak rise, %	Time, min	Peak rise, %	Time, min
1	17	50	20	65
2	18	55	18	60
3	17	60	16	60

[2] Sollman, T. H., and Hanzlik, P. J., *Fundamentals of Experimental Pharmacology*, J. W. Stacey Co., San Francisco, 1939, 241.

[3] Hardy, J. D., Wolff, H. G., and Goodell, H., *J. Clin. Invest.*, 1940, **19**, 649.

Antipyretic Action in Rabbits. An elevation in body temperature was produced in 8 rabbits by injecting intravenously 1 cc of a preparation of killed *Eberthella typhosa* containing approximately 10^7 organisms/cc. Rectal temperatures were measured before the injection and at intervals afterward. Either the monoanilide of aconitic acid or acetanilide was administered orally to groups of rabbits after an elevation of 2-4 degrees in body temperature was noted, usually 2 hours after the initial injection. The resultant decreases in temperature are compared in Fig. 1 with the temperatures of a group of rabbits given no drug.

Summary. The monoanilide of aconitic acid appears to have no advantages over acetanilide as an analgesic or antipyretic agent. The activity of the monoanilide seems to be related to the aniline in the molecule, as is the case with acetanilide.[4] No additional pharmacological actions were noted which could be ascribed to the propene group of the aconitic acid.

[4] Michel, H. O., Bernheim, F., and Bernheim, M. L. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 321.

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Starch Reaction as Aid in Identification of Causative Agent of "European Blastomycosis."

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The clinical picture and symptomatology of "European Blastomycosis" ("Torulosis") is variable, and unreliable as a basis of diagnosis. The differentiation of the etiological agent is beset with difficulties, and its cultural and morphological resemblance to contaminating non-pathogenic yeasts has given rise to much confusion in the literature of yeast infections. The diagnostic scheme outlined below may be helpful in this connection, especially to pathologists without training in mycology, as it avoids the use of refined morphological criteria.

While studying the biochemical properties of nonfermenting capsulated yeasts, we observed that a strain isolated from a case of torula-meningitis produces extracellular starch in special growth conditions.[1] The amount of starch produced is considerable and can easily be detected macroscopically when a plate culture of the organism is flooded with Lugol's iodine solution.

We have now extended our investigation

[1] Aschner, M., Mager, J., and Leibowitz, J., *Nature*, 1945, **156**, 295.

to 16 other strains of this pathogenic yeast and in all of them starch formation was observed in the growth conditions indicated below. The individual strains varied as to the intensity of starch production. In the majority of cases a positive reaction for starch could be obtained after 24 - 48 hours of growth. In some strains the formation of starch was weaker and delayed for 5-8 days. A large collection of other yeast genera was tested and none of them exhibited the phenomenon of extracellular starch production. A substance which stains blue with iodine may be found in cultures of Schizosaccharomyces,[2] but only intracellularly and in very limited amount. The production of extracellular starch seems to be specific for a group of nonfermenting capsulated yeasts of which *Torulopsis neoformans* is a representative. This group includes also nonpathogenic species[3] which can, however, be differentiated by testing the growth optimum. Benham has shown, that whereas pathogenic *Torulopsis neoformans* (*Cryptococcus hominis,* according to Benham's nomenclature) thrives at 37°C, nonpathogenic species of this group show optimum growth at 30°C and poor or no growth at 37°C.[2]

In the light of these findings we arrive at the following method for the identification of *Torulopsis neoformans:*

Yeasts isolated from cases suspected of blastomycosis should be transferred to a synthetic medium composed as follows:

$(NH_4)_2SO_4$	0.1%
$MgSO_4$	0.05%
KH_2PO_4	0.1%
Glucose	1%
Thiamine	0.2 μg per ml
Agar-agar	2.5%

Ammonium sulfate serves in this medium both as a source of nitrogen and as a regulator of the acidity necessary for production of starch.[1] Vitamins other than thiamine are not essential for cultivation of starch-producing yeasts in synthetic media.

After some days of incubation at 37°C, the plate is flooded with Lugol's iodine solution. In positive cases the whole streak of growth usually turns a deep blue. If, however, the amount of starch elaborated by the yeast is small, the reaction is perceptible only in isolated spots and after excess of iodine has disappeared. The extracellular character of the starch may be ascertained by examining with iodine the color of the agar beneath a growth-streak after the cells are scraped off from the agar surface. An alternative method is to cultivate the yeast in a liquid medium (composed as above), where optimal growth can be obtained by aeration or continuous shaking. After the cells have been separated by centrifugation, the supernatant is tested for starch.

According to our results, ability of extracellular starch formation combined with unimpaired growth between 37° and 40°C are features characteristic enough to classify a nonfermenting yeast as *Torulopsis neoformans* (Sanfelice) Lodder.[4,5]

Summary. A method for the identification of *Torulopsis neoformans* (syn. *Torula histolytica, Cryptococcus hominis*) is described.

The method is based on the ability of this organism to produce extracellular starch in special growth conditions.

We should like to express our thanks to Prof. Kluyver, Dr. Lodder, and Dr. Benham for their kindness in sending us a large number of different yeast cultures.

[2] Beijerinck, M. W., *Centralblatt f. Bakt. u. Parasitenk.*, 1897, **3**, 449.

[3] Benham, R. W., *J. Infect. Dis.*, 1935, **57**, 255.

[4] Lodder, J., 1934, *Die Anascosporogenen Hefen*, p. 152, Amsterdam.

[5] Lodder, J., *Mycopathologia*, 1938-9, **1**, 62.