YEASTS, PROBABLY PATHOGENIC, IN THROAT CULTURES *

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The finding of yeast-like organisms in smears from throat cultures has been reported from time to time by observers, some of whom have considered them to be the cause of pseudomembranous angina, while others have considered them to be secondary contaminations, of no pathological significance.

In 1893 Troisier and Achalme[1] mentioned finding yeast-like organisms in a case of membranous angina. In 1897 De Simoni[2] obtained such organisms directly from 5 cases of hypertrophied tonsils. In a study of 500 cultures from patients suspected of having diphtheria De Stoecklin[3] found yeasts in 37 cases. In 1899 Foullerton[4] carefully worked out the cultural and pathogenic characteristics of various yeasts, two of which were from cases of membranous angina. He proposed the name Saccharomyces tumefaciens albus because culturally they gave white growths and when inoculated into guinea-pigs, caused swellings at the site of inoculation.

In 1901 Bertarelli and Calamida[5] found yeast-like organisms in a large percentage of individuals who themselves were not ill, but had associated with others that had membranous angina. Wilson[6] reported 136 cases of membranous angina in which B. diphtheriae was absent. In most of these cases the yeasts were present in almost pure growths. In the 12 cases which he had seen personally, the tonsils were congested, swollen, and showed a rapidly developing, thin grayish-white membrane. He also found yeasts associated with B. diphtheriae in 97 cases. In all instances his organisms gave a white growth, which was thick and moist, and which in fluid media fell to the bottom of the tube in a few days. They all fermented maltose and glucose, but not lactose. Breed[7] has also reported diphtheroid tonsillar membranous disease due to yeasts.

About 3 years ago my attention was directed to the seemingly large proportion of throat cultures in routine examinations that showed yeast-like organisms. From time to time such cultures were plated out and examined in considerable detail. As there was no attempt to

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1 Arch. de méd. expér. et d'anat. path., 1893, 5, p. 29.
2 Centralbl. f. Bakteriol., 1897, 22, p. 120.
3 Arch. de méd. expér. et d'anat. path., 1898, 11, p. 1.
4 Jour. Path. and Bacteriol., 1899, 6, p. 37.
5 Centralbl. f. Bakteriol., I, O., 1901, 30, p. 60.
6 St. Paul Med. Jour., 1904, 6, p. 649.
7 Arch. Int. Med., 1912, 10, p. 108.

examine every culture of this kind, I have no statistics as to the percentage showing yeasts. In all, 90 cultures were used. Some had been sent into this laboratory for the purpose of obtaining either a diagnosis of diphtheria or a release from quarantine. Many of them

TABLE 1

PHYSICAL CHARACTERISTICS OF CULTURES

No.	Glucose Agar	Plain Agar
1	Confluent, white, moist, glistening, and heaped up. Later, yellowish-green	A thin white film.....
2	Dull-white, dry, flat, and confluent.....	Dull-white, dry, flat, and confluent
3	Confluent, white, very moist, glistening, and heaped up. Later, yellowish-green	Rather scanty, confluent, moist, white
4	Confluent, dry, dull, cheesy, whitish, later becoming a lemon yellow	Scanty, moist, glistening, white.....
5	Very white, heaped up, dry, and glistening. Later pink	Discrete, heaped-up, white, star-shaped colonies
6	Confluent, heaped up, moist, glistening, salmon colored, and later yellow	Confluent waxy growth; along streak, pale-yellow
7	Confluent heaped-up cheesy mass, drab-white in old cultures	Confluent, moist, translucent, dirty-white
8	A confluent heaped-up growth like yellow paint. White in old cultures	Same as glucose agar.....
9	Rather scanty confluent dry yellow growth	Thin transparent yellowish film.....
10	Confluent, pink, moist, and glistening. Later dirty-gray	Same as on glucose agar.....
11	Confluent, salmon-gray, moist, paint-like along streak. Later yellow and finally white	Same as on glucose agar.....
12	Confluent, like streak of white paint. Later greenish-yellow	Same as on glucose agar only drier
13	Scanty white film, later becoming yellow, and finally brown	Scanty white growth.....
14	Confluent white veil-like growth with abrupt edges; dry partly rose-tinted	Same as on glucose agar.....
15	Confluent flat grayish growth, flows over surface of medium	Dry white film.....
16	Like bright-red paint along streaks, moist and shining. Later a gray thin film	Same as glucose agar.....
17	Like a streak of bright-yellow paint. Later grows as discrete pale-yellow colonies	Same only drier.....

Under glucose agar, the chromogenesis described as a later appearance refers to that observed after 5 subcultures had been made.

The 56 yeasts recovered are divided into the 17 varieties shown in this table as follows:

were from wholesale examinations in schools where diphtheria was epidemic.

Smears were made in the ordinary way and stained with Loeffler's methylene blue. These were kept for comparison. Then the culture

TABLE 1—*Continued*

PHYSICAL CHARACTERISTICS OF CULTURES

Broth	Gelatin Stab	Potato
White pellicle with cuff up sides of tube	Slight growth along stab, brown	Slight, dull, confluent, dry, grayish-white
White pellicle with cuff up sides of tube	White discrete colonies along stab and heavy white growth on surface	Scanty, confluent, dull-white
At first white pellicle, later this falls to bottom. Fluid becomes clear	Slight yellowish growth along stab	A moist confluent whitish growth
Pellicle at first; later media settles clear	Slight growth along stab. Near surface, white	Dry, dull, confluent, white
Media cloudy. Slight precipitate	Same as No. 4.....	Confluent, moist, shining yellow
Media clear. Deep-brown pellicle at top	Yellowish-brown, heaped up on surface, slight along stab	Moist, glistening, yellowish-pink, confluent
White precipitate at bottom. Media clear	White on surface. No growth along stab	A heavy confluent moist white growth
Media clear; slightly brown pellicle and precipitate	Heavy growth along stab and at surface; brown	A heavy confluent salmon-colored growth
Media cloudy; brown precipitate; slight pellicle	Yellow mostly at surface	Confluent, dry, yellow
Media clear; brown precipitate; slight pellicle	White mostly at surface	Confluent, moist, shining, and gray
Media cloudy; white pellicle and white precipitate	Heavy gray growth at surface and along whole stab	Confluent gray white growth
Media clear; white precipitate and pellicle	Growth white on surface, scanty along stab	White confluent moist growth
White precipitate; medium cloudy	Very scanty white growth along stab	Scanty moist white growth
Media cloudy; white precipitate and pellicle	Slight liquefaction; brownish along stab	Confluent wet creamy growth
White precipitate; medium clear	Discrete white colonies along stab	Thin white growth, dry
Media clear; brown precipitate and pellicle	Slight pink growth along stab	Brown along streak
Media clear; yellow precipitate and pellicle	Heavy yellow growth on surface and along stab	Bright-yellow, moist, glistening, and heaped up

11 under No. 1, 4 under No. 2, 4 under No. 3, 2 under No. 4, 4 under No. 5, 4 under No. 6, 3 under No. 7, 2 under No. 8, 1 under No. 9, 2 under No. 10, 2 under No. 11, 2 under No. 12, 4 under No. 13, 3 under No. 14, 2 under No. 15, 5 under No. 16, and 2 under No. 17.

was plated out and the colonies obtained were examined. If there appeared to be yeasts, the colonies were transferred to glucose agar. From this they were grown on all the usual laboratory media, including 9 kinds of sugar-broth media in fermentation tubes.

Table 1 shows the results on ordinary media and Table 2 shows the results with special media. In only 56 instances was it possible to recover an organism that appeared to be a yeast. No organism that fermented inulin was considered to be a yeast. In 3 cases an organism of the oidium group was recovered; in 2 cases, leptothrix; in 20 cases, a mold only was recovered, and in 9 instances no fungus was recovered.

TABLE 2

REACTIONS ON SPECIAL MEDIA

Culture No.	Dextrin	Dextrose	Galactose	Lactose	Levulose	Maltose	Mannite	Raffinose	Saccharose	Glycerin	Inulin	Indol	Nitrites
1	—	+ Gas	+	+	+	—	+	+	—	—	—	+	—
2	—	+	—	—	—	—	—	—	—	+	—	+	+
3	—	+	+	+	—	—	—	—	—	—	—	—	—
4	—	+ Gas	—	—	+ Gas	—	—	—	+	+	—	—	—
5	+	+ Gas	+	—	—	—	+	+	+	—	—	—	—
6	+ Gas	+ Gas	+ Gas	+ Gas	+ Gas	+ Gas	+ Gas	—	+ Gas	+	—	—	—
7	—	+	+	—	+	+ Gas	+	—	+	+	—	+	—
8	+ Gas	+	+ Gas	+	+ Gas	+ Gas	+ Gas	+ Gas	+ Gas	+	—	+	—
9	—	+	+	—	+	+	—	—	—	+	—	—	—
10	—	+	+ Gas	—	—	—	—	—	+ Gas	—	—	—	—
11	+	—	—	—	—	—	—	—	—	—	—	—	—
12	+	+	+	—	+	+	—	—	+	+	—	—	—
13	+	+ Gas	+	+	+ Gas	+	—	—	+	—	—	+	—
14	—	+	—	—	—	—	—	—	+	+	—	—	—
15	—	+ Gas	+	+	+ Gas	—	—	—	—	—	—	+	+
16	+	+	—	—	+	—	—	—	—	—	—	+	—
17	+	+	+	—	+	+	—	+	+	+	—	—	—

+ = acid-formation in fermentation tube or production of indol and nitrites.
— = no change.

It is interesting to note here the recovery of a mold in such a large number of cases. However, it is well known that certain molds have a yeast-like stage, particularly the penicillium group. I have found by comparing the original smears that it is impossible to detect any difference between those that gave yeasts on the plates and those that gave molds. The question whether or not these molds have any pathological significance, has been put aside for future work.

In all, 17 distinct varieties have been recovered, as shown in Table 1.

It is still a debated question as to the importance of color in a culture of a yeast. By referring to Table 1 it is seen that often the organism changes color with a change of medium. Then, again, as in No. 14, we get two colors at the same time. Lafar[8] mentions certain

TABLE 3

CLINICAL FACTS CONCERNING CASES FROM WHICH CULTURES WERE OBTAINED

Case	Number in Table 1	Laboratory Diagnosis as to Diphtheria	Remarks
1	1	+	Not obtained
2	2	—	School contact
3	3	—	Contact
4	4	+	Prolonged convalescence
5	5	—	See history of Case 1
6	6	+	Prolonged convalescence
7	7	—	Contact
8	8	—	Contact
9	9	—	Not obtained
10	10	—	Not obtained
11	11	—	Not obtained
12	6	—	Contact
13	11	—	School contact
14	14	+	Patient, a carpenter, had been repairing an old church. Convalescence very long continued. Secondary abscess
15	13	—	Contact
16	14	—	Not obtained
17	15	—	Contact
18	16	—	Tonsillitis
19	17	—	School contact
20	5	—	School contact
21	7	—	Tonsillitis
22	14	—	Contact
23	16	+	Uneventful
24	13	—	Contact
25	16	—	School contact
26	6	—	Contact
27	6	—	Contact
28	16	—	Contact
29	14	—	Contact
30	1	—	Contact
31	1	—	Pharyngitis
32	1	—	Contact
33	7	+	Delayed convalescence
34	16	—	Contact
35	2	+	Delayed convalescence
36	10	+	Enlarged tonsils with very slow recovery
37	1	—	See history of Case 2
38	5	—	See history of Case 3
39	17	—	See history of Case 4
40	5	+	Enlarged tonsils with secondary abscess
41	4	—	See history of Case 5
42	2	—	Not obtained
43	1	—	School contact
44	3	+	Uneventful
45	8	—	Tonsillitis
46	3	—	Contact
47	3	—	Tonsillitis
48	1	—	Not obtained
49	1	—	Secondary abscess
50	1	—	Not obtained
51	12	—	Uneventful
52	15	+	Not obtained
53	13	—	See history of Case 6
54	2	—	Contact
55	13	—	Contact
56	1	—	See history of Case 6

[8] "Technical Mycology," 6, II.

yeasts with variations in color. If we ignore this point and depend on cultural characteristics alone, the question of classification is much simplified. Also, it is not yet absolutely proved that the formation of gas in sugar media is constant with the individual yeast.

Morphologically, yeasts vary a great deal in shape and size, according to the conditions under which they are kept. Practically all work for the purpose of classifying yeasts has been done by botanists, who have based their classifications almost wholly on morphology and physical characteristics. In the present paper no attempt has been made to classify the organisms recovered or to apply to them the specific names already proposed by former workers. This work, however, is being attempted and will be published at a later date.

None of the individuals from whom the cultures were obtained has been seen clinically by the writer, but, in most cases, the clinical history has been obtained from the attending physician. Table 3 gives these clinical details briefly.

The following histories referred to in Table 3 are of interest:

CASE 5.—Woman, school-teacher, aged 20 years. Illness began Nov. 14, 1914, with a sore throat. Other members of the family had had the same trouble a short time before, and a sister who teaches in the same school had sore throat at about the same time. It was prevalent also among children in this school. The patient showed some prostration, and had a fetid breath. Headache. Two large dark-gray membranous patches almost covered each tonsil. Pulse 120, temperature 102 F. Five hundred units of antitoxin were given. All cultures negative. Returned to work Nov. 23. On Nov. 27 the woman developed scarlet fever as did several other members of her family.

CASE 37.—Woman, who had suffered from tonsillitis for from 12 to 15 years. Illness began January 13, with a sore throat. Temperature 101, pulse 96. Both tonsils swollen and inflamed, but no exudate present. Cultures negative. The case yielded readily to ordinary treatment. Patient apparently well on 15th. On the 17th she developed a post-tonsillar abscess, which was evacuated. Recovery.

CASE 38.—Girl, aged 16. Sudden prostration with severe headache. Temperature 104.6, pulse 120, respiration 26. Two days later a small white membrane formed in the throat; very firm and difficult to remove. Culture negative. There was considerable albumin in urine. Some edema. Recovery slow.

CASE 39.—Man, taken ill Dec. 6, 1914, with chills, pain in back, and headache. Temperature slightly elevated. A post-tonsillar abscess was located and drained, after which patient recovered.

CASE 41.—This patient was a man who lived in the same household as the patient in Case 37. He presented swollen and inflamed tonsils, a temperature of 101 and a pulse of 110. Two days later, left tonsil was incised and pus evacuated. Recovery immediately followed. Cultures negative.

CASE 53.—Child, 4 years old. Temperature 101, pulse 86, respiration 24. Cervical glandular enlargement. Breathing labored and very croupy. Fetid

breath. Four days later, cheesy spots on both tonsils. Breath more fetid. Next day silvery white membrane covering both tonsils. Cultures negative. Given 4000 units of antitoxin without result. Membrane by next day had filled both nostrils, throat, and larynx. Child had to be intubated. Repeated cultures and smears were all negative. Under steam and local treatment membrane gradually disappeared in 4 days. Very little toxemia during attack.

CASE 56.—A brother of the patient in Case 41, aged 8 years. Illness started in the same way, but after the membrane had extended to the nostrils, it began to disappear. All cultures negative. Six thousand units of antitoxin given. Child not confined to bed.

From the data in Table 3 we see that 23 of the cultures were from contact cases, contracted either from suspected carriers in schools or from other cases of diphtheria in the same household. No significance at present can be given to these cases. It may be found that these yeasts are present in a large proportion of all normal throats. We do know that yeasts, as demonstrated in the case of blastomycetes, are very common in old buildings. The only instance that I have seen in which the yeasts in the throat may have come from an old building concerns a carpenter who had been repairing an old church, in whom diphtheria developed followed by a slow convalescence, yeasts of Group 14 being found in the cultures.

Ten of the cases had the yeasts associated with B. diphtheriae; in 7 of these we find tonsillar or other abscesses, or a prolonged convalescence, so that it really seems as if the yeasts do complicate the course of diphtheria. Whether these symbiotically render B. diphtheriae more resistant cannot yet be decided. It appears also that a large proportion of cases with infection and inflammation of the throat show yeasts when one would naturally expect B. diphtheriae; indeed, the evidence is almost conclusive that a pseudomembranous disease can be excited by these organisms. It is also noteworthy that tonsillar abscesses were present in so large a number of cases in which the yeasts were either found alone or associated with B. diphtheriae.

The 17 varieties of yeasts have all been inoculated subcutaneously in guinea-pigs. In every case, except Nos. 3, 5, 8, and 15, a general glandular enlargement resulted. The one inoculated with No 14, died in 3 weeks with extreme emaciation. No pathologic changes other than the glandular enlargement could be found. A yeast culturally the same as the one inoculated was recovered from the glands. The animal inoculated with No. 1 developed an abscess of one of the cervical glands, the pus of which gave a pure growth of a yeast culturally similar to the one inoculated. One other animal was killed—the one

inoculated with No. 9—and a yeast recovered from one of the glands culturally the same as the one inoculated. The other animals were still alive, about 2 months later, and still showed glandular enlargement.

In another series of guinea-pigs the inside of the cheek was scarified, and one of the cultures rubbed over the area. In all these animals, except those inoculated with Nos. 3, 5, 8, 11, 15, and 16, after about 48 hours there developed a dirty-yellowish false membrane that in the case of those inoculated with Nos. 1 and 17 extended down over the mucous membrane of the cheek, and down into the throat. The others had the membrane only over the area abraded. This membrane, which stripped very easily, on cultivation gave a yeast apparently the same as that which had been inoculated.

In conclusion, it may be said that yeasts are found in the throats of a certain percentage of all individuals, and that under proper conditions they become pathogenic, setting up either an inflammation of the mucous membranes of the throat and air-passages, or else producing a more deeply seated infection, such as a tonsillar or peritonsillar abscess. There must be long continued research before one shall be able to tell how many varieties of yeasts may be pathogenic or even to classify the present known varieties as to exact species. Experimentally, most of these organisms appear to be pathogenic for guinea-pigs. From a study of the clinical histories, I believe them to be pathogenic for man. However, as before stated, more work will have to be done before this point can be proved.

SUMMARY

This work has demonstrated that yeasts are a factor in the production of so-called throat infections, either alone or in association with diphtheria. It seems also that when the latter condition is present convalescence is either unusually prolonged or complicated.

The cultural characteristics of the organisms recovered from throat cultures have been worked out in considerable detail, but as yet no specific classification has been attempted.

The organisms for the most part are pathogenic for guinea-pigs both when locally applied to the abraded mucous membrane and when injected subcutaneously.