SOME CLINICAL AND EXPERIMENTAL OBSERVATIONS WITH A SACCHAROMYCETE *

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INTRODUCTION

My object in presenting this paper is to relate the facts in connection with my observations and to call attention to the possible significance of yeast organisms in the sputa of doubtful lung cases.

I am greatly indebted to the physicians of Pomona who have so kindly and generously cooperated with me in these investigations, and to Dr. Charles C. Browning of Los Angeles for consultation and suggestions in Case 7; to Dr. D. J. Davis of St. Luke's Hospital, Chicago, for his interest and valuable suggestions, and also to Dr. I. C. Herb of the Rush Medical College for assistance in translation of literature. I desire to express my deep appreciation for the ever ready and helpful interest shown by Dr. Stanley P. Black of Pasadena, whose wise counsel and advice have been a constant incentive to careful work.

LITERATURE

Claude Bernard,¹ the French physiologist, experimenting with yeast in 1848, produced infections in animals. Since that time various observers have had similar results, using for their experiments wine yeasts, beer yeasts and the thrush fungus. Sanfelice,² experimenting in 1895 with two varieties of saccharomyces, one from fruit juice, found them to be pathogenic for guinea-pigs and white mice. Lydia Rabinowitch³ made a careful experimental study with forty yeasts which were obtained from different sources. She found eight pathogenic, some producing granulomatous nodules and others a septicemia. Maffucci and Sirleo⁴ found that a species of saccharomyces produced in guinea-pigs a pulmonary affection which resembled tuberculous pneumonia. Demme's⁵ *Saccharomyces ruber*, which he held responsible for an outbreak of intestinal catarrh in a family of seven children, was found to be pathogenic for guinea-pigs, dogs and mice.

That saccharomycosis of the mucous membrane is possible has been demonstrated by Colpe⁶ and Bosdike, who obtained a pathogenic yeast

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1. Bernard, Claude: *Leçons de Pathologie Experimentale*, 1871.
2. Sanfelice, Francesco: *Centralbl. f. Bakteriöl.*, 1895, xviii, 52.
3. Rabinowitch, Lydia: *Ztschr. f. Hyg. u. Infektionskrankh.*, 1895, xxi.
4. Maffucci, A., and Sirleo, L.: *Policlinico*, 1895, p. 138.
5. Demme, R.: *Berlin* 1890, Hirschwald.
6. Colpe: *Arch. f. Gynäk.*, 1894, xlvii.

from a patient with chronic catarrh of the uterine cervix. Busse⁷ found the fungus in proliferative catarrh of the nasal mucosa. Busse also, in 1894, described a yeast which he found in a case of pyemia and which he named *Saccharomyces hominis*. In all he published four communications regarding this fungus, and the same organism was described by Buschke.⁸ Gilchrist⁹ described a pure skin disease caused by a yeastlike fungus. Curtis¹⁰ published an account of a case in which a yeast caused myxoma-like tumors. He called this fungus *Saccharomyces humane*. Huebner¹¹ found a saccharomycete in pustules of the skin on various parts of the body. Corselli and Frisco¹² cite a case in which nodules were seen in omentum and mesentery. In this case yeast organisms were found in chylous and ascitic fluids obtained by exploratory puncture during

Fig. 1.—Saccharomycete in fresh drop. Oil immersion.

life, and were found to be pathogenic for guinea-pigs, rabbits and dogs. Troisieur and Achalme¹³ found a saccharomycete in a case of pseudo-membranous angina in a typhoid fever patient. Clinton Hickey¹⁴ of

7. Busse, Otto: Ueber Saccharomycosis hominis, Virchows Arch. f. path. Anat., 1895, cxl, 23; Experim. Untersuch. über Saccharomycosis, Virchows Arch. f. path. Anat., 1896, cxliv, 360.

8. Buschke, A.: Klin. Vorträge, 1898, No. 218.

9. Gilchrist, T. C.: The Johns Hopkins Hosp. Rep., 1896, i, 269.

10. Curtis: Contribution à l'Etude de la Saccharomycose humaine, Ann. de l'Inst. Pasteur, 1896, x, 449.

11. Heubner: Deutsch. med. Wehnsehr., 1904, Nos. 33 and 34.

12. Corselli, G., e Frisco, B.: Centralbl. f. Bakteriöl., 1895, xviii, 368.

13. Troissier, E., and Achalme, P.: Arch. de méd. expéd. et d'anat. path., 1895, v, 29.

14. Hickey, Clinton: Colorado Med. Jour., 1900, vi, No. 2, p. 485.

Denver described in 1900 two cases of scarlatinal angina and one of "sore throat" in which a dense exudate covered tonsils, pillars of fauces and margins of soft palate. The city bacteriologist reported "no Klebs-Löffler bacilli, no streptococci, and no staphylococci, but numerous yeast cells." Steinhaus¹⁵ found a yeast in a child suffering from scarlet fever which he called *Saccharomyces membranogenes*. On the fifth day the

Fig. 2.—*Saccharomycete*, smear from 24-hour culture, stained with Löffler's methylene-blue. Oil immersion.

Fig. 3.—*Saccharomycete*, smear from 3-weeks-old culture showing many empty capsules, stained with Löffler's methylene-blue. Oil immersion.

child developed croup with a very fetid odor. On the twenty-first day dyspnea developed and tracheotomy was performed. After operation the child coughed up a tenacious mass, following which breathing became

15. Steinhaus: Centralbl. f. Bakteriolog., I abt. Orig., 1906-07, xliii, part 1, p. 49.

easier. An aseptic instrument was passed into the trachea and membrane secured for cultures. One thousand units of antitoxin were given, as diphtheria had been diagnosed. The patient died next day. Autopsy was not allowed. Cultures were made on Löffler's blood-serum. Sixteen hours later the plate showed large, glistening, round, light yellow-tinged colonies which, much to his surprise, revealed pure cultures of yeast.

Fig. 4.—*Saccharomycete* in tissues, surrounded by leukocytes, mostly polynuclears; hematoxylin and eosin stain. Oil immersion.

Fig. 5.—*Saccharomycete*, nuclear stain, Janssen's and Leblanc's modification of Mueller's method. Oil immersion.

In his animal experiments, mice, guinea-pigs and rabbits were employed. He used intravenous, subcutaneous and abdominal injections; also feeding bread soaked in bouillon cultures of yeast, all of which produced the characteristic infection, and at autopsy the characteristic miliary tubercles

and sections revealed the yeast fungus in all organs and in blood-vessels and nerves. His article is complete in every detail. Reitman¹⁶ cites a case, a patient of 30 years, sick eleven days, who died four days after entrance to the hospital. The autopsy showed croupous pneumonia and glomerulitis. Only sections of kidneys were examined, but many of these sections contained double-contoured refractile bodies between 5 and 20 microns found in epithelium of the tubules in the glomeruli, and free in the blood-stream. It was impossible to make cultures in this case on account of the non-recognition before hardening. He depended on staining and morphology, but thinks that they correspond to the organism described by Busse. Castellani,¹⁷ director of the Clinic for Tropical Diseases, Colombo, gives some interesting observations on fungi found

Fig. 6.—Saccharomycete; smear from pus in Case 14; nuclear stain for differentiating. Oil immersion.

in tropical bronchomycoses, to which various names are given (tea-factory cough, copra cough, etc.). Among other fungi he found a saccharomycete twice.

The subject of pathogenic yeasts has been so carefully and thoroughly covered, and the literature so completely reviewed by Ricketts in his monograph "Oidiomycosis (Blastomycosis) of the Skin and Its Fungi," and by Hektoen, in his summary of cases of "Systemic Blastomycosis and Coccidioidal Granulomata," that further reference except to these two

16. Reitman: *Centralbl. f. Bakteriol.*, 1895, p. 225.

17. Castellani, Aldo: Observations on the Fungi found in the Tropical Bronchomycoses, *Lancet*, London, 1912, i, No. 1, p. 13.

works, with their bibliography, seems unnecessary. Hektoen¹⁸ says that the history of many of the cases cited by him, and the bronchopneumonic character of some, may be taken to indicate their air-borne nature. In a number of these cases blastomycetes were found in the sputum, and in some of them the pulmonary symptoms were the first to appear, followed in most cases by localization of lesions.

Regarding the occurrence of yeasts in Nature, Klocker¹⁹ says:

"Hansen's researches on the circulation of saccharomycetes in Nature, and on the amount of microorganisms in the air at various seasons of the year, have led to the following results, very important to the brewer: (1) Wind and insects are the most important means of transportation of yeast cells in Nature, especially the first. (2) Dust clouds in the harvest months are rich in strong yeast cells produced on sweet, juicy fruits." He describes saccharomycetes as "single cell fungi in which vegetative increase takes place by budding and which develop endospores in their interior under certain conditions; sometimes they may form typical mycelium. The various related species are grouped according to their action on various sugars."

In a careful comparative study of other known cells, together with the yeast plants, Mutchler came to the following conclusions which he thought justifiable: (1) Yeast-cells contain structural elements that are homologous with the structural elements of the cells of higher plants and with the known structural elements of animal cells and with the structural elements of the bacteria and the cyanophyceæ as worked out by Butchle and also by Kumbler. (2) The structural elements consist of four definite regions, are found in both mature cells and in the growing buds and correspond to what is well known in the higher plants and in animal cells as cytoplasm, nuclear membrane, nucleus and nucleolus. The structural elements may be demonstrated clearly by the four methods of differential staining discussed by him and are to be found in every yeast cell. Emerson²⁰ says that as the presence in the sputum of certain pathogenic yeasts cannot be denied, in doubtful cases they should be looked for as a possible explanation of anomalous lung conditions. Hektoen suggests further careful study of new cases both clinically and anatomically, and further observations in regard to the blood, especially to the number of leukocytes and differential counts. B. F. Davis²¹ of Chicago has given valuable information in immunological reactions of oidiomycosis.

18. Hektoen, Ludvig: Jour. Am. Med. Assn., 1907, xlix, 1071.

19. Klocker: "Fermentation organisms," 1903. English translation of the German edition, p. 248.

20. Emerson, Chas. P.: Clinical Diagnosis, 1911.

21. Davis, B. F.: Jour. Infect. Dis., 1911, viii, 190.

AUTHOR'S OBSERVATIONS OF A YEAST

During more than two years of clinical laboratory work in Pomona I have encountered a yeast in fifteen individuals sent to me for laboratory diagnosis. This organism I first discovered in a vaginal discharge. Four months later I found similar organisms in the sputum of a child said to have tuberculosis, and again in a culture from the tonsil membrane of a child supposed to be suffering from diphtheria. The fourth time I encountered this fungus was in the sputum of a patient with a pulmonary lesion resembling lobar pneumonia in the early stage of resolution. In this case repeated examinations of the sputum failed to reveal any tubercle bacilli and the tuberculin test was negative. To ascertain what was causing the symptoms I made cultures from the sputum and each time got a pure growth of an organism which I identified as a saccharomycete and which gave the same results culturally and with animal experimentation as did the fungus found in the vaginal discharge in the first case.

After the above experience I made a routine practice of washing and examining culturally all sputa sent to the laboratory in which, after repeated examinations, I found no tubercle bacilli. As a result I have discovered a yeast organism in a number of other patients which corresponds morphologically and culturally to those found in the preceding cases. At times the organism was found in pure culture, again in connection with a staphylococcus or *Micrococcus catarrhalis*, and twice it was found mixed with tubercle bacilli, but in all cases the morphological and cultural characteristics were identical, and animal experiments with the strains tested gave the same results.

Morphology.—This organism resembles the *Saccharomyces cerevisiae* both culturally and morphologically, but the latter on all culture media grows less profusely and is less spherical. It is about the size of a red blood-corpuscle, possesses a double-contoured capsule and contains fine granules and refractile bodies like fat or vacuoles. Old cultures show lessening of granules and extension of vacuoles, and numerous empty capsules. It grows by budding and possesses a nucleus which can be demonstrated by special staining. In one old culture there was a slight tendency to form threads. I have found no endospores but have made no special cultures for spore formation.

Staining Properties.—In smears from cultures this organism stains well with all ordinary dyes. It is Gram positive. It is not acid fast. Direct smears from pus or thin sputum are best treated with Wright's blood stain, which colors the pus cells a pinkish purple and the yeast organisms a dark blue, while the red blood-cells are a brick red. For staining the nucleus I have used Janssen's and Leblanc's modification of Mueller's method as used by brewers, the technic of which is given in "Klocker's Fermentation Organisms." This requires four days but is

excellent for differential purposes. It stains the nucleus a dark red and the cytoplasm a pale pink.

Cultural Characteristics.—On glucose and glycerin-agar slants there is a profuse creamy growth at 37 C. in twelve to twenty-four hours. On potato slants but a slight film in the same length of time. It turns litmus milk a full creamy white in twenty-four hours with formation of a soft curd. It grows vigorously on both plain and glucose bouillon, forming a heavy sediment in the bottom of the tubes, and in the latter the appearance of foam on shaking. There is a profuse growth on both plum and grape decoctions but no foam on shaking the tubes. It does not liquefy gelatin, but growth appears along the line of the stab with roseate formation at the top. Agar plates reveal white moist points in twenty-four hours. On all culture media there is but slight growth at room temperature. The minimum temperature for visible growth of this organism is 18 C. and the maximum temperature is 69 C. It is devitalized at 71 C. for one hour. Two-year-old cultures which were quite dry were moistened with salt solution, and from them fresh culture media inoculated. A fairly vigorous growth resulted. Glucose is fermented, giving rise to alcohol and carbon dioxid. Growth occurs in lactose, dextrose, raffinose, inulin and mannite in 2 per cent. solution, though there is no evidence of fermentation. On all culture media this organism gives rise to the characteristic odor of yeast. Plates continually exposed in the laboratory reveal no growth of this fungus.

Animal Experiments.—This organism is pathogenic for rabbits, white rats, guinea-pigs and monkeys. Subcutaneous injections produce slowly developing septic conditions from which the animals tend to recover if not injected too often. Intraperitoneal injections produce in guinea-pigs and white rats a rapidly fatal septicemia; in rabbits and monkeys within a few weeks rapid loss of weight and more slowly developing septicemia. In all animals within a few hours after intraperitoneal injections there is rapid breathing, high temperature and some leukocytosis with slight increase of mononuclear cells. Autopsies without exception reveal grayish miliary tubercles thickly studding all internal organs, and peritoneal and pericardial surfaces. Sections of these tubercles show masses of the yeast-cells, surrounded by leukocytes, mostly polynuclears. The organism was recovered in pure culture from the heart's blood, bile, peritoneal and pericardial fluids and the miliary tubercles, and direct smears showed full-grown and budding yeast cells.

HISTORIES OF CASES IN WHICH THE ORGANISM OCCURRED

CASE 1.—Physician, Dr. Huntington; patient, J. H., aged 11. The patient came under observation Dec. 4, 1909. Four months previously while swinging on a rope she lost her hold and slid down to the knotted end, receiving injuries about

the external genitals. When first seen the labia were red, smooth, shining and sore, small whitish patches thickly covering the parts. These lesions which, when first seen, were confined to the external genitals, later invaded the vaginal tract. The whitish patches rapidly coalesced and formed a clinging membrane, and if not treated frequently the underlying parts became highly inflamed and very sore. When daily treatments were given, cultures showed feeble growth; but if three or four days elapsed between treatments, cultures yielded vigorous growth. From December, 1909, until March, 1910, daily and often twice daily treatments were given. From March until July laboratory examinations were continued at intervals, but during this period there was no reappearance of the patches, or growth on culture media, although the parts remained red and shining. In July the characteristic whitish patches were again observed on the inflamed parts and a culture taken revealed a profuse growth of the yeast in twelve hours. It was a problem to find a solution of anything strong enough to affect the growth without irritating the mucous membrane. Weak solutions of mercury chlorid, copper sulphate, ichthyol and boric acid all caused pain. Various powders proved inefficient. Cleaning the parts with sterile water, using gentle friction, gave best results. Douching the parts with sterile water without friction had no effect. The patient had little systemic affection. The cervical glands were slightly enlarged, but there was no elevation of temperature; the patient gained in weight during the treatments. Late in July the family removed to Oregon and two months later the father of the child reported that there was no further trouble.

CASE 2.—Physician, Dr. Toland; patient, a 4-year-old son of Mrs. T. The mother in April, 1910, consulted Dr. Toland about the child, who was coughing excessively. This case had been pronounced tuberculosis by her former physician. In the sputum sent to the laboratory for examination I found a yeast, but no tubercle bacilli. The mother left town soon afterward and the child was not heard from again until December, 1911. The cough was still present, but the child had improved physically.

CASE 3.—Physician, Dr. Toland; patient, W. M. S., business man, aged 36. The patient came under notice in 1910 with pharyngitis. He had lost in weight slightly and feared tuberculosis. The tuberculin test was negative; repeated examinations of sputum covering a period of several months failed to reveal tubercle bacilli. Cultures from sputum yielded pure growth of a yeast. The cough continued to be troublesome for more than a year but by November, 1911, it had entirely disappeared.

CASE 4.—Physician, Dr. Savage; patient, a girl of 10 years, with a history of chronic enlargement of the tonsils for two years, which, when first seen, were soft and boggy, associated with acute inflammation of the entire throat, and dense yellow patches confined to the tonsils. From a detached piece of membrane sent to the laboratory a culture revealed a vigorous growth of a yeast but no Klebs-Löffler bacilli. While waiting for the laboratory diagnosis antitoxin was given, but the membrane continued to form and three weeks later intubation was performed. The child made a good recovery.

CASE 5.—Physician, Dr. Swindt; patient, Mr. H. V. S., aged 56, a rancher. This patient was first seen in June, 1910. Two weeks before coming under notice he had a severe "cold" and cough, with viscid, blood-streaked sputum. At the time of entrance to the hospital he had chills, sweats and a temperature of 102 F. At the base of the left lung were detected signs of lobar pneumonia undergoing resolution. The blood and urine were normal. The tuberculin test was negative, and frequent examinations of the sputum revealed no tubercle bacilli, but cultures from the sputum resulted in a pure growth of a yeast. Large doses of sodium iodid were given and within two weeks the pulmonary symptoms had entirely disappeared. Phlebitis of the left leg occurred while in the hospital, and lasted for about three weeks. The patient reported, Jan. 15, 1912, that there was no cough or other pulmonary symptoms. The left leg swelled some when he worked hard.

CASE 6.—Patient, M. H., servant girl, aged 22. This patient was sent directly to the laboratory for a sputum examination by her employer, on account of a constant cough. She had come to California during the early summer on account of "catarrh." Her general health improved but the cough persisted. Repeated examinations of sputum failed to reveal tubercle bacilli. The tuberculin test was negative and the blood was normal. Cultures from the sputum yielded an almost pure growth of a yeast. Her employer advised her to seek another position and she took service in a mountain resort, but the high altitude did not agree with her and she went to Long Beach. Information from her first employer in November, 1911, was that she had entirely recovered.

CASE 7.—Physician, Dr. Browning; patient, Mrs. E. D. C., aged 35, married. The patient came from Idaho to southern California on account of persistent cough which dated from an attack of bronchitis in March, 1910, from which time she complained of a pressure over the chest and a difficulty in breathing. She had a loose paroxysmal cough with a profuse mucopurulent expectoration which was discharged after violent effort. There was a moderate degree of emaciation. Temperature was 99 F., pulse 100. The appetite was good and the digestion fair. The right side of the chest was normal, with dulness over entire left chest and especially pronounced over the third rib. Sputum examinations at intervals of from two to four days from January until May, 1911, failed to reveal tubercle bacilli. The tuberculin test was negative. Frequent cultures from the sputum always yielded a pure growth of a yeast and in a blood culture (bouillon) the same organism was found. Autobacterins of the yeast were given at seven-day intervals for six weeks, also various preparations of the iodids. The patient gained in weight and improved markedly, and in cultures from the sputum the growth of the yeast organism gradually disappeared. April 23 she took a long auto ride, during which she became overtired and thoroughly chilled. This resulted in an elevation of temperature (104 F.) and great prostration, and thereafter tubercle bacilli were observed in every specimen of sputum examined. As soon as the patient was able to travel again she went east and died six months later.

CASE 8.—Physician, Dr. Davis; patient, Miss V. H., aged 19, a student. In June, 1909, this patient came under observation with a laryngitis which did not yield to treatment. Examination of the lungs revealed a small area of infiltration, in which occasional moist râles were heard, just external to left sternoclavicular articulation. The morning temperature ranged from 96.6 F. to 100.2 F. Afternoon temperature ranged from 101 F. to 104 F. There was a troublesome cough and a profuse expectoration; also gradual loss of weight and night sweats. In November, 1909, a sputum examination revealed tubercle bacilli mixed with pus organisms. Tuberculin was not given until December, 1910, from which time this treatment was continued until March, 1911, but the temperature remained high and the patient was losing ground. At this time the sputum was sent to my laboratory with a view of having an autobacterin prepared for the mixed infection. Sputum examinations revealed very few tubercle bacilli. Cultures revealed a luxuriant growth of a yeast, with *Staphylococcus aureus*. The tuberculin was discontinued and an autobacterin of the yeast organism given every seven days for six weeks. The afternoon temperature gradually but steadily decreased but never at any time became normal. During the same time the yeast growth decreased until there was scarcely anything visible on culture media in twenty-four hours. The patient's general health, however, did not improve and she died May 29, 1911. There was no autopsy.

CASE 9.—Patient, Miss V. R., school girl, aged 16. This patient was almost a constant companion of the 16-year-old daughter of the patient, Case 7. She was sent directly to the laboratory by her mother for a sputum examination because of a constant, distressing cough. A sputum examination revealed no tubercle bacilli and the tuberculin test was negative. In a culture from the sputum pure growth of a yeast resulted. Potassium iodid, 10 grains three times daily, caused the cough to disappear entirely within a few weeks.

CASE 10.—Patient, Mrs. R., seamstress, aged 51, mother of patient Case 9, developed a cough soon after the daughter. All of the findings were exactly as in Case 9, but the cough was more persistent and she was obliged to keep up the iodids longer and in much larger doses. Eight months later both patients were entirely well.

CASE 11.—Physician, Dr. Swindt; patient, Mr. C. H. A., clerk, aged 55. This patient had a chronic bronchitis for thirty years, but his general health was good up to five years previous to examination. During the previous five years he had occasional fever and frequent attacks of hemoptysis, but never any marked loss of weight. He had profuse fetid expectorations for the previous four years. There were signs of chronic bronchitis in both lungs. The fetor disappeared under sodium iodid, and creosote reduced the amount of expectoration. In April, 1911, a blood examination was negative, a tuberculin test negative and there were no tubercle bacilli found. A culture from the sputum revealed a yeast with *Staphylococcus albus*. An autobacterin of the yeast organism caused an exacerbation of symptoms which alarmed the patient and the dose was not repeated. Patient went east in May of the same year. A few months later he reported that he was taking staphylococcus-vaccine (stock) without improvement. This patient had frequent sputum examinations during the past five years and tubercle bacilli were never found. An unidentified organism, however, had been reported once.

CASE 12.—Physician, Dr. Swindt; patient, Mrs. J. B. P., aged 67. This patient had chronic cough for many years. During the previous four years she suffered greatly from paroxysms resembling pertussis. Her general health was good. Both lungs showed signs of moderate bronchitis. No tubercle bacilli, but pure culture of a yeast from the sputum. The paroxysms were completely relieved and the cough reduced to a minimum in about three weeks by the use of sodium iodid in large doses.

CASE 13.—Physician, Dr. Garcelon; patient, W. S., aged 47. This patient was in the hospital with typhoid fever, and in a culture from the feces, in connection with the typhoid bacilli, a yeast was found. The patient died after a two months' illness. No autopsy. As this yeast organism resembled all of the preceding ones morphologically and culturally, animal experiments were undertaken with it, and the same results obtained as in the previous cases. It was also identified as a *saccharomycete*.

CASE 14.—Physicians, Dr. Huntington, Dr. Swindt; patient, Miss L. T., aged 35. The patient came under observation in November, 1910. She had had arthritis deformans for ten years and the spine and large joints were stiff. Four years previously she had appendicitis, but no operation. Three years previously she had pleurisy with effusion, followed by empyema and evacuation through the left bronchus. Since then there had been a constant, profuse expectoration. At intervals of three to eight weeks there would be an enormous increase of this expectoration, when, in a short time, from 1 to 3 pints of stinking pus would evacuate through the bronchus, pouring through nose and mouth and almost strangling the patient. In February, 1911, a sputum examination revealed no tubercle bacilli, but a culture showed a yeast, together with *Staphylococcus albus* and *M. catarrhalis*. Nov. 7, 1911, a sputum examination gave the same results as ten months previously, but with an increase of the yeast organism. The tuberculin test was negative; there was some leukocytosis with 82 per cent. polynuclears. The temperature ranged from subnormal to 103 F.; pulse from 90 to 120; respiration from 18 to 26. A radiograph showed a shadow on the left side below the clavicle. November 11 the seventh and eighth ribs on the left side were resected and over 3 pints of pus removed. The lung was found in a small, contracted mass high up in the cavity, with apparent communication with the bronchus. The condition of the patient did not permit of decortication. The cough disappeared and the general condition immensely improved following the evacuation of pus. In smears from the pus from the wound yeast organisms were found. Culture from the pus revealed yeast organisms in almost pure growth. An

agglutination test of the yeast with this patient's serum, 1-40 and 1-50, was strongly positive, while controls were negative. An extract of yeast made for me in the Cutter Laboratory, Berkeley, according to the technic for Koch's old tuberculin, proved slightly positive in a skin test, controls negative. Autobacterins of the yeast were begun a few weeks after the patient left the hospital and her improvement has been uninterrupted.

CASE 15.—Physician, Dr. Kelly. The patient, Miss A. C., aged 15, school girl, had always been subject to "colds." She came under notice in December, 1911, with a severe cough. The mother of the child said that cough and expectoration had been continuous for the previous three months. There was some loss of weight and some anemia. The temperature was 99 F., pulse 90; blood examination showed hemoglobin 50 per cent., red blood-cells 80 per cent., no leukocytosis, polynuclears 83 per cent.; tuberculin test negative. An examination of the sputum every day for two weeks revealed no tubercle bacilli. A culture from the sputum showed a vigorous growth of a yeast with *Staphylococcus aureus*. An agglutination test with the yeast was slightly positive, controls negative. The patient refused to have a skin test with the yeast extract. Large doses of sodium iodid were given.

The above histories are not complete, but abstracts as given me by the physicians in whose service they occurred.

NOTES ON CASES

In few of the cases herein cited have we had opportunity for careful clinical study. Only two of the patients were in the hospital at all and then for only a short time. The organisms from Cases 1, 4, 5, 7, 8, 13, 14 and 15 were employed in animal experiments with practically the same results. In Case 1 we were not allowed to make a skin test or blood examinations, but the clinical picture was observed closely. In taking smears or cultures here, the parts were always cleansed first with sterile water. It was significant that with the disappearance of the yeast fungus in this case the trouble disappeared entirely. Case 2 we had on opportunity of observing. Case 4 suggests cases cited by Hickey and Steinhaus in the literature, inasmuch as a tenacious membrane formed and reformed, and that there was an absence of the Klebs-Löffler bacillus. As Case 5 was in the hospital I made frequent blood and sputum examinations, but at that time had not begun making the skin and agglutination tests with the yeast. However, the absence of any organism except this fungus suggested the iodids. There was no opportunity to observe Case 6 after the laboratory analysis and it was only through the kindness of her first employer that we learned of her present condition. In Cases 7 and 8 tubercle bacilli were found mixed with the yeast organisms, but it would seem to be a secondary invader in the former, while probably in the latter the tuberculous infection was primary. Both of these patients died, but as there was no autopsy in either case, there can be no definite knowledge regarding this. It is, however, significant that both of these patients were benefited by autobacterins and iodids. The facts in Cases 9 and 10 suggest that this organism may be transferred

from one person to another. The suspension of yeast organisms prepared for Case 11 was kept at a temperature of 70 C. for one hour, as the previous ones for Cases 7 and 8 had been, and as a twenty-four-hour culture taken from this emulsion was sterile, the dose was then given. After forty-eight hours, however, the culture tube revealed a slight growth of yeast. This undoubtedly accounted for the exacerbation of his symptoms.

Case 12 was remarkable in that it was an unmixed infection of yeast, and that it was so quickly and entirely relieved by the iodids. Case 13 was not observed with regard to the yeast organism found. We isolated and identified it simply because it resembled the organisms previously identified as *saccharomycetes*. We had more opportunity for careful study of Case 14, and the observations were fairly satisfactory. The very positive agglutination test, the slightly positive skin reaction, together with the occurrence of the same organism in the pus from the wound that I had previously and repeatedly found in the sputum, are suggestive of the pathogenicity of the *saccharomycete* in this case. Case 15 had only begun to improve with the iodids when she began taking a patent medicine and was lost sight of. In all of these cases the physicians have remarked the unusual fetor and viscid character of the sputum and repeated attacks of "colds."

The examination of the sputum requires some care. It should be washed in normal or tenth-normal sodium or potassium hydrate, and cultures made from the parts resembling pus. Smears should be treated as described above. If the sputum is to be examined unstained it should be covered with a normal solution of sodium or potassium hydrate, and allowed to stand for at least thirty minutes to dissolve the mucus. The yeast cells being resistant to the action of the alkali can easily be found and are not confused with red blood-cells.

This report leaves much to be desired in the way of clinical observation of cases, and autopsy records in cases of death; also blood examinations, especially in cases in which the *saccharomycete* is the only organism found. The complement deviation test should also be made in connection with the agglutination and skin tests. Animals should be immunized and agglutination tests made with their sera.

Five other patients are still under observation in whom a yeast has been found. One is a child aged 4½ years with repeated attacks of bronchitis followed by asthma. It has been impossible thus far to secure a satisfactory specimen of sputum, as she swallows it, but a yeast has been found in a blood culture (agar plate), also in a culture from the feces during one of her attacks of bronchitis, and her serum agglutinates the yeast organism. A skin test has not yet been made. An old lady of 79 has had ulcers on parious parts of her body for eight months past.

Yeast cells were found in the pus from one of these ulcers and could easily be recognized both in unstained and stained specimens. The remaining three patients have a severe cough, and a prominent feature is the profuse fetid expectoration and the frequent attacks of "cold." We are hoping for something more definite from the study of these cases.

SUMMARY

A yeast which has been identified as a saccharomycete has been observed in the sputum of a number of patients with anomalous lung conditions, also in a tonsil membrane, a vaginal discharge and in pus from a skin abscess. It was mixed with other organisms in most cases, but has been found as the only apparent cause of infection in a few patients in whom disappearance of the organism has been followed by alleviation and disappearance of the symptoms.

Autobacterins made from this yeast organism have seemed to cause some improvement in three cases and an exacerbation of symptoms in one.

The most benefit has been derived from the use of the iodids in large doses. An extract prepared from this saccharomycete gave a slight reaction in a skin test on two patients. The serum of four patients gave a positive agglutination test for the yeast.

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