Theme of this year II

FUNGUS PROBLEMS IN OTOLARYNGOLOGY

By

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Since Langenbeck found thrush to be a fungus disease, it has been well known that fungi have some relations with ear, nose, mouth and throat. In the otolaryngological area, however, studies on fungi and fungus diseases are very few, much less than those on bacteria and bacterial diseases.

In the present paper the summary of the author's studies on fungus problems in otolaryngology is to be described. The studies have been carried out in Formosa and Japan since about 20 years ago.

I. Mycological Flora of Ear, Nose, Mouth and Throat

This investigation was made in the whole year of 1955 in Kyoto, Japan. The materials were normal healthy persons and out-patients not suffering from any mycotic disease. cultures from 843 ears of the same number of persons, 812 noses, 806 mouths and 819 throats were all mycologically thoroughly examined.

Tabl 1 shows the sorts of fungi found as well as number of each fungus; for *Aspergillus* and *Candida* the names of species were given, while for most of other fungi the names of genus were reported and for some others mere family names or order names were mentioned.

Candida was encountered most frequently (188 cases), *Penicillium* was the second (150 cases), *Aspergillus* the third (150 cases) and *Hormodendrum* the fourth (106 cases). *Alternaria* (30 cases), *Papularia* (34 cases), aerobic Actinomycetaceae (40 cases), Phomaceae (25 cases) and Mycelia sterilia (61 cases) were fungi found relatively often.

The frequency of fungus-positive cases was somewhat different according to the organs: ear 28.0%, nose 30.3%, mouth 25.8% and throat 30.3%. While there was no significant difference between ear and nose, the frequency of the nose is considerably higher than that of the throat. This difference between nose and throat can be explained by the filtrating function of the nose: the majority of fungus spores in the air inhaled through the nostrils might be caught by the mucus of the mucous membrane and, therefore, the air coming into the throat contains much less fungus spores than that in the nose itself.

The frequency of each fungus was also different according to the organs as

Table 1

Fungus	Ear	Nose	Throth	Mouth	Total
Actinomycetaceae	15	15	4	6	40
Phycomycetes					
<i>Mucor</i>	2	2	1	1	6
<i>Cunninghamella</i>		1			1
<i>Syncephalastrum</i>	1	1	1		3
Ascomycetes					
<i>Chaetomium</i>	1	1			2
Basidiomycetes					
<i>Ustilago</i>	1	1	1		3
Fungi imperfecti					
Phomaceae	15	5	2	3	25
Melanconiaceae					
<i>Pestalozzia</i>	1		1		2
Mucidinaceae					
<i>Malbranchea</i>	1				1
<i>Monilia</i>		1			1
<i>Geotrichum</i>				1	1
<i>Oidium</i>		3	1		4
<i>Gliocladium</i>				1	1
<i>Penicillium</i>	28	85	19	18	150
<i>Paecilomyces</i>		1			1
<i>Scopulariopsis</i>	9	6	2		17
<i>Dactylomyces</i>		1			1
<i>Cephalosporium</i>	2	2			4
<i>Trichoderma</i>	2	3	5		10
<i>Verticillium</i>	2				2
<i>Sporotrichum</i>	1	2	1		4
<i>Monosporium</i>	2	2			4
<i>Acremonium</i>	1	1			2
<i>Aleurisma</i>	2	1	1	1	5
Dematiaceae					
<i>Torula</i>	2	3		1	6
<i>Hormiscium</i>	2	3	1	1	7
<i>Echinobotryum</i>		1			1
<i>Catenularia</i>	1				1
<i>Dematium</i>	3	3	1	1	8
<i>Pullularia</i>	5	3	2		10
<i>Hormodendrum</i>	26	37	8	9	80
<i>Stachybotrys</i>	1				1
<i>Trichobotrys</i>		1			1
<i>Phialophora</i>			1		1
<i>Nigrospora</i>	5	1	2	2	10
<i>Hemispora</i>	1				1
<i>Monotospora</i>	4				4
<i>Acremoniella</i>	1	1			2
<i>Septonema</i>	1				1
<i>Heterosporium</i>					1

<i>Helminthosporium</i>		1			2
<i>Alternaria</i>		17	7	3	30
<i>Stemphylium</i>		6	7		14
Tuberculariaceae				1	
<i>Fusarium</i>		1	2		5
<i>Papularia</i>		21	9	3	34
Stilbaceae				1	
<i>Stisanus</i>			3		3
<i>Mycelia sterilia</i>		19	25	7	61
<i>Aspergillus</i>	Total	39	44	11	106
<i>Asp. repens</i>			3		4
<i> ruber</i>		1	1		2
<i> proliferans</i>		2			3
<i> chevalierii</i>			1		1
<i> amstelodami</i>		1			1
<i> conicus</i>			1		1
<i> restricus</i>		1	3		4
<i> penicilloides</i>		1	2		3
<i> fumigatus</i> v. <i>rugophorus</i>		1		1	2
<i> unguis</i>		1			1
<i> paranidulans</i>			1		1
<i> sydowii</i>		5	7	3	16
<i> sydowii</i> v. <i>microvesicularis</i>		2		1	3
<i> sydowii</i> v. <i>procerus</i>			1		1
<i> sydowii</i> v. <i>rugophorus</i>			1		1
<i> versicolor</i>		7	6	2	18
<i> versicolor</i> v. <i>rugophorus</i>			1		3
<i> terreus</i>		1			1
<i> candidus</i>			1		1
<i> niger</i>		1	2	1	4
<i> awamori</i>			2		2
<i> fumarius</i>			1		1
<i> wentii</i>		1			1
<i> oryzae</i>		1	1	1	3
<i> oryzae</i> v. <i>microsporus</i>					1
<i> oryzae</i> v. <i>magnosporus</i>		1			1
<i> flavus</i>		11	6	2	24
<i> ochraceus</i>		1			1
<i> ochraceus</i> v. <i>microsporus</i>			1		1
Yeasts					
Endomycetaceae		1		1	4
Cryptococcaceae				2	
<i>Torulopsis</i>		19	18	29	91
<i>Candida</i>	Total	6	4	79	168
<i>Candida albicans</i>		1	1	49	115
<i> tropicalis</i>		2	1	9	24
<i> krusei</i>				1	2
<i> parapsilosis</i>		1	1	5	11
<i> pseudotropicalis</i>				1	3

<i>guilliermondii</i>	1		4	2	7
<i>pelliculosa</i>			2	2	4
<i>scottii</i>			1	2	3
<i>solani</i>			1	4	5
<i>reukauffii</i>			1	2	3
<i>Trichosporon</i>	1		1	1	3
<i>Rhodotorula</i>	11	4	1	3	19

Table 2

	Ear	Nose	Throat	Mouth
Number	843	812	819	806
<i>Aspergillus</i>	39	44	11	12
%	4.68%	5.14%	1.34%	1.49%
<i>Penicillium</i>	28	85	19	18
%	3.36%	10.47%	2.32%	2.23%
<i>Hormodendrum</i>	26	37	8	9
%	3.12%	4.54%	0.98%	1.12%
Yeasts	37	27	117	152
%	4.44%	3.33%	14.3 %	18.9 %

shown in Table 2 for four main fungi: *Aspergillus*, *Penicillium* and *Hormodendrum* were observed more frequently in ear and nose than in mouth and throat, while yeasts were encountered much more frequently in mouth and pharynx than in ear and nose. This datum might show that most yeasts found in mouth and throat are to be regarded as normal inhabitants there.

Aspergillus, *Penicillium* and *Hormodendrum* failed to show any seasonal and age variation, on the contrary, yeast were found much less in winter and spring than in summer and fall, and they were observed more rarely in the second and third decades than in the other age groups.

II. Otomycosis

The incidence of Otomycosis is much higher in the Far East than in Europe and North America. The author found the disease in Formosa as the rate of 7.02% of all ear patients, while he and his collaborators got the rate of about 2% in Japan. Literatures in Europe and North America show much less rates (0~0.5%).

As predisposing factors, climatic conditions, age, scratching of the ear canal and water in the auditory canal (after swimming, bathing or shampoo) should be taken into consideration.

The climate of Japan is generally warm and humid and in Formosa it is very hot and more humid. After the author's assumption, the high incidence of otomycosis there may be chiefly due to these climatic conditions. Even in the two countries the disease is observed far many in hot and humid summer and few in cold or cool winter: in a clinic in Formosa the author found averagely 173 cases of otomycosis in August and 17 in February. The correlation coefficient between the number of otomycosis patient (p) and the temperature (t) was $r_{pt} = +0.753$, and the partial correlation coefficient between them, the influences of the humidity (h) and the wind-velocity (w) being excluded, was $r_{p \cdot hw} = +0.533$.

The susceptibility to the disease is higher in older ages than in younger. Although the actual number of otomycosis cases was the smallest in the group of older age over 70 years and the largest in the young of 20~29 years, in the ratio to the population of each age group there was a corresponding increase of the disease with the advancement of age.

The scratching or digging in the ear canal was found as a predisposing cause in 80% of the otomycosis cases.

The intrusion of water into the external auditory canal (swimming, bathing, shampoo etc.) was mentioned in 43.5% in Formosa and in 50.0% in Japan.

The quality of cerumen failed to have relation with the incidence of otomycosis: the disease was found in 2.3% of the persons with moist (sirupy, honey-like, soft or wax-like) cerumen, while in 2.4% of the persons with dry (scaly, membrane-like or hard) cerumen. It must be here mentioned that in Japan and Formosa the moist cerumen, popular in the people of the Western Countries, is observed only in about 10% of the population and that the greatest majority (90%) has dry cerumen.

The sorts of fungi found in the otomycosis cases in Formosa and Japan as well as the number of cases of each fungus are tabulated in Table 3, comparing with those appeared in the literatures of the foreign countries, chiefly of Europe and North America.

Aspergillus is the most prevalent everywhere in the world, also in Japan and Formosa. *Scopulariopsis* and *Candida* are found far less than *Aspergillus*, and other fungi are very rare (Table 4).

As for the species of *Aspergillus*, those of *A. terreus* group were found most frequently in Japan and Formosa (about 60%), while they are very few in the Western Countries (about 5%). On the contrary, *A. niger* was found in only 4% in Japan and Formosa, though it is prevalent in the Western Countries (54%).

At present in U.S.A. a skepticism against "Otomycosis" rules over the oto-

Table 3

	Foreign Countries		Japan		Total
	Formosa Yamashita	others	Yamashita Hikita	others	
<i>Mucor mucedo</i>		2			2
<i>Mucor</i> sp.		4			4
<i>Lichtheimia ramosa</i>		1			1
<i>L. corymbifera</i>		5			5
<i>L. maligna</i>	4				4
<i>L. italiana</i>		1			1
<i>Rhizomucor</i> sp.		1			1
<i>R. parasiticus</i>		1			1
<i>Rhizopus</i> sp.		1			1
<i>Aspergillus glaucus</i> gr. sp.		18			18
<i>A. montevidensis</i>		1			1
<i>A. chevrierii</i>	1				1
<i>A. malignus</i>		1			1
<i>A. penicilloides</i>	2				2
<i>A. fumigatus</i>		64		7	71
<i>A. fumigatus</i> v. <i>magnus</i>	46				46
<i>A. fumigatus</i> v. <i>formosanus</i>	64				64
<i>A. fumigatus</i> v. <i>major</i>	2				2
<i>A. fumigatus</i> v. <i>rugophorus</i>			9		9
<i>A. sydowii</i>	9		2	4	15
<i>A. sydowii</i> v. <i>microvesicularis</i>	3				3
<i>A. sydowii</i> v. <i>procerus</i>	7				7
<i>A. versicolor</i>				1	1
<i>A. versicolor</i> v. <i>thermophilus</i>	7				7
<i>A. versicolor</i> v. <i>levis</i>	7				7
<i>A. flavus</i>		57	7	19	83
<i>A. oryzae</i>		1	24	4	29
<i>A. soyae</i>		3			3
<i>A. jeanselmei</i>	2				2
<i>A. niger</i>		185	9	5	199
<i>A. macfiei</i>		1			1
<i>A. awamori</i>	9		6		15
<i>A. wentii</i>				6	6
<i>A. terreus</i>	34	2	67	34	137
<i>A. terreus</i> v. <i>pauper</i>	28		39		67
<i>A. terreus</i> v. <i>albidus</i>	172		15		187
<i>A. terreus</i> v. <i>pallidus</i>	33		14		47
<i>A. venetus</i>		1			1
<i>A. candidus</i>	3	3		8	14
<i>A. albus</i>		2			2
<i>A. ochraceus</i>		2		1	3
<i>A. ochraceus</i> v. <i>microsporus</i>				1	1
<i>A. melleus</i>				4	4
<i>A. tamari</i>				8	8

<i>A. nidulans</i>		5	4	9
<i>Asp. sp.</i>		13	64	77
<i>Penicillium minimum</i>		1		1
<i>P. epigaeum</i>		2		2
<i>P. bicolor</i>		1		1
<i>Peni. sp.</i>	7	6	24	37
<i>Scopulariopsis blochii</i>	21			21
<i>S. blochii v. alba</i>	6			6
<i>S. brevicaulis</i>			2	2
<i>S. humilis</i>	5	1		6
<i>S. nivalis</i>	7			7
<i>S. divaricata</i>		10	5	15
<i>S. divaricata v. alba</i>		3		3
<i>S. sasakiana</i>			1	1
<i>Scop. sp.</i>		2	20	22
<i>Paecilomyces sp.</i>	4			4
<i>Candida formosana</i>	2			2
<i>C. tropicalis</i>	2	2		4
<i>C. taiwana</i>	7			7
<i>C. otica</i>	5			5
<i>C. otica v. petasata</i>	3			3
<i>C. otica v. pulveraris</i>	3			3
<i>C. rhoi</i>		1		1
<i>C. parapsilosis</i>			2	2
<i>C. krusei</i>			1	1

laryngological circle. In the cases that had been clinically regarded by them as "otomycosis", they have found mostly *Pseudomonas aeruginosa* and rarely fungi. Therefore the otologists in America have the tendency to consider that, even if fungi were found in the auditory canal, they should not be true pathogenic agents but be mere contaminations or secondary invaders to any other disease.

The skepticism, however, is a fault and groundless one, at least for true, typical otomycosis properly diagnosed. This is thoroughly clarified by the author's studies as follows.

1) If the fungi of true, typical "otomycosis" were not pathogenes but only contaminants, or secondary invaders, remarkably many kinds of fungi (over 40 genera) that can be found in the normal external meatus as shown in Table 1

able 4

	Europe	America	Far East (Japan & Formosa)
<i>Aspergillus</i>	198 94.3%	63 75 %	746 83.7%
<i>Scopulariopsis</i>	0	2 2.4%	83 8.7%
<i>Candida</i>	0	6 7.1%	33 3.5%
others	12 5.7%	13 15.4%	39 4.1%
Total	210	84	949

5) The author and his collaborators have succeeded in making the experimental otomycosis in rabbit ears and in human ears.

In rabbits, *Aspergillus fumigatus* var. *magnus*, *A. fumigatus* var. *major*, *A. fumigatus* var. *rugophorus*, *A. terreus*, *A. terreus* var. *pauper*, *A. terreus* var. *albidus*, *A. terreus* var. *pallidus*, *A. penicilloides*, *A. candidus*, *A. niger*, *Scopulariopsis fuliginosa*, *Lichtheimia maligna* and *Candida taiwana* were employed as experimental fungi; and, in human ears, *A. fumigatus* var. *magnus*, *A. terreus*, *A. terreus* var. *pauper*, *Scopulariopsis fuliginosa*, *Lichtheimia maligna* and *Candida taiwana*.

After thoroughly cleansed and disinfected, the external meatus was scratched gently and one of the fungi above mentioned was introduced. In human cases otomycosis occurred in 27 of 39 ears experimented and in rabbits more frequently. The otomycosis occurred experimentally resembled very much to that of clinical patients. They showed the characteristic membranes in the meatus with some inflammation, and in human cases sense of itching and fullness were complained of. They healed readily with phenylmercuric acetate and, when they were neglected to treat, they often recurred. Observing histologically it showed an inflammation of the epithelium and the dermis, the formation of the mycotic membrane and sometimes the invasion of fungal hyphae into the epithelial layer (Fig. 1, 2, 3). Culturings readily revealed the introduced fungi.

Prior to our experiments, Carnevale-Ricci, Italy, and Africa, C. M., Philippines, reported to have succeeded in making experimental otomycosis, the former in rabbits and the latter in monkeys. In human ears, however, the author and his collaborator are the first who have ever succeeded in the formation of experimental otomycosis.

In this way the postulate of Robert Koch has been completely established and there remains no doubt about the pathogenicity of some fungi to otomycosis.

The first step to the diagnosis of otomycosis is to keep the fact always in mind that the disease is not very rare and that, in humid and warm or hot countries, it is rather often to be encountered.

The symptoms such as itching, fullness and deaf are general in otomycosis, but "frequent accumulation of large ceruminous membrane", that is in reality the mycotic membrane but is called "large cerumen" by patients in Japan and Formosa, is the symptom of otomycosis observed most frequently, 86.4% in Japan and 83.2% in Formosa.

The most important objective sign is the mycotic membrane confined to the deep portion of the osseous canal and or to the tympanic membrane. It consists of desquamated epithelial layers, more or less mycelia of fungi grown in

and or the layers, infiltrated leucocytes and some secretions. In the most characteristic cases the membrane makes a hollow cast of the auditory canal (having a form like a whisky glass). Such was observed in about 15% of otomycosis cases in Japan and Formosa. Even if it is not so peculiar as that above mentioned, the membranes often takes a form of socks. About two-thirds or three-fourths of otomycosis cases in Japan and Formosa are the dry type with one or little secretion. In such type the membrane often adheres to the underlying tissue so firmly that it can not be removed by wiping or syringing, while in the moist type the membrane is free in the meatus or attached loosely to the meatal wall.

For making diagnosis of the disease, it is absolutely necessary to find the fungus vegetation on or in the membrane. We have not ever made a diagnosis of otomycosis without finding fungi on or in the membrane, while in America, this indispensable procedure seems to be very often neglected or omitted.

There are two routine methods to verify fungi on or in the membrane, the dry mount and the wet mount.

If the case is the dry type, the membrane taken out from the auditory canal is placed on a slide (when it takes a form of cast or socks, it must be cut and stretched to make a plane membrane) and without adding any mounting fluid and also without covering it with a cover-glass, it is examined under the microscope. In this case one must use the natural light falling on the specimen and never use the light through the mirror and the condensor. Then one can readily find beautiful, cobweb-like mycelia and sometimes fructification organs such as conidiophores and conidial heads.

If the case is the moist type, a mounting fluid should be dropped on the specimen on the slide, and then the membrane is cut into smaller pieces arranged with needles and covered with a cover-glass. This is examined in the usual way of microscopy, utilizing the light through the mirror and the condensor. As mounting fluid, the author found that 10% KOH with cotton blue 0.1% was the best.

Culturing and identification of the fungus is also important for diagnosis of otomycosis, especially, when the diagnosis is not determined by clinical observations with the dry or wet mount microscopy.

If the fungus taken from an otomycosis case is identified to be a species of *Aspergillus terreus* group, *A. fumigatus* group or *A. niger* group, the diagnosis is assured, because such fungi are the most important and frequent ones as fungi of otomycosis and because they are very much rare in the ear of normal persons or in other ear diseases. When *Aspergillus flavus*, *A. sydowii* or *A. ver-*

sicolor is identified, one must be very careful for regarding it as the causing fungus, because those three species are sometimes discovered in normal ears. The other species of *Aspergillus* are much more doubtful. *Scopulariopsis* and *Candida* are somewhat significant. Many other genera than *Aspergillus*, *Scopulariopsis* and *Candida* have much less or none value as the causing fungus of otomycosis.

For the treatment of otomycosis very many kinds of drug have been used since last century.

The author and his collaborator have studied in vitro the fungicidal and fungistatic power of 183 kinds of drug or formula, including almost all experienced or recommended for otomycosis in the past, new disinfectants, deys, agricultural drugs for fungus diseases of plants and antibiotics. Fungi examined were 15 strains obtained by the author from the exact otomycosis cases in Formosa and Japan, their pathogenicity being verified experimentally in rabbits and human ears.

Based on the results of the present three kinds of test, the 183 drugs or formulae were classified into seven classes.

Iodine tincture (containing iodine in 6.3%), 0.1% phenylmercuric acetate (aqueous solution), and 5% silver nitrate (aq. sol.) belonged to AAA class; 0.02~0.03% phenylmercuric acetate (aq. sol.), 1% mercuric chloride (in alcohol) and 5% carbolic acid (ap. sol.) to AA class; 2% thymol (in abs. alcohol), 0.2% mercuric chloride (in alcohol) and other ten kinds to A class; 70~98% alcohol, 0.1% mercuric chloride (in alcohol), 1% thymol (in alcohol), 2% salicylic acid (in alcohol) and other twenty-two to B class; metaphen tincture, 50% alcohol, 2% thymol (in 70% alcohol) and other twenty to C class; 10% carbolic acid (in glycerol), undecilenic acid and other nine to D class; 5% carbolic acid (in olive oil), 1% thymol (in water), 2% thymol (in olive oil), 2% thymol (in glycerol), 1% lysol (aq. sol.), cresatin, 5% mercurochrome (aq. sol.), 2% methyl violet (aq. sol.), 1% gentian violet (aq. sol.), 1% crystal violet (aq. sol.), furacin, streptomycin, penicillin, chlormphenicol, aureomycin, terramycin, ilotycin and about seventy more belonged to E class.

Almost all drugs of AAA and AA class have such unfavorable nature that they have irritative effect and make the patient feel pain or burn in the ear when applied, especially on the case having a perforation of the tympanic membrane. In AAA and AA class, the aqueous solution of phenylmercuric acetate alone has no irritative effect. These were clearly testified by the author's experiments dropping those drugs into the eyes and ears of rabbits.

Therefore the aqueous solution of phenylmercuric acetate should be rega-

ded as the best one for the treatment of otomycosis.

The author and his many collaborators have applied 0.02% phenylmercuric cetate in water for otomycosis since over 15 years and in more than 1000 cases. We drop it in the ear and let it stay there for ten to fifteen minutes, once a day in a week. The present method has always satisfied us, because it has completely improved the disease without exception and without any recurrence. Moreover, it has not any by-effects and fails to make patients feel any pain or burn in the ear.

III. Otitis media and Fungi

Several years ago the author found some cases of chronic otitis media non-cholesteatomatosa that showed marked resistance against usual treatments with antibiotics and were revealed to contain yeasts in the secretion. The cases were satisfactorily treated with a fungicide, phenylmercuric acetate. Therefore, a systematic survey on the relation of fungi to chronic otitis media considered to be desirable.

A series of examination of 155 cases of chronic otitis media noncholesteatomatosa revealed that some 9% of them contain yeasts in them. The yeasts obtained were classified as follows; *Rhodotorula mucilaginosa* var. *carbonei* 1, *Candida albicans* 2, *C. tropicalis* 3, *C. solani* 2, *C. utilis* 2, *C. parapsilosis* 3, *C. eukaufii* 1, *C. guilliermondii* 1, *Saccharomyces* sp. 1 and *Ustilago* sp. (yeast form) 1.

It is likely to be a significant matter that in chronic otitis media other fungi than yeasts could hardly be found, though there are innumerable sorts of fungus in the external auditory canal as well as in the pharynx.

Some cases of chronic otitis media having yeasts with them show a peculiar symptom, gelatin- or agar-like, half-solid secretion that can be hardly removed by syringing, suction or wiping with cotton applicator. The cases with such secretion had tendency to resist strongly against antibiotics and could be cured only by the fungicide. The author assumes that in those cases yeasts should have played a certain role in the course of the disease.

IV. Sinusitis and Fungi

In Japan and Formosa chronic sinusitis is very common, possibly much more than in the Western Countries. It is one of the author's suppositions for explaining the popularity of the disease and its resistance against the routine therapy that fungi should be playing a role in it.

Mycological examination of pus obtained from the maxillary sinus revealed that fungi were frequently found (about 40%) in the diseased sinus.

The sort and number of fungi found were as follows: *Aspergillus versicolor* 10, *A. humicola* 3, *A. sydowii* 2, *A. sydowii* var. *columnaris* 2, *A. amstelodami* 1, *A. repens* 3, *A. ruber* 3, *A. bicolor* 2, *A. candidus* 2, *A. atrofuscus* 1, *A. sulphureus* 1, *Penicillium* 27, *Hormodendrum* 20, *Mycelia sterilia* 9, *Scopulariopsis* 4, Actinomycetaceae 1, *Candida* 1, *Fusarium* 1, *Papularia* 6, *Alternaria* 3, Phomaceae 8, *Gliocladium* 1, *Cephalosporium* 1, *Mucor* 3, *Epicoccum* 1, *Trichoderma*, *Torulopsis* 2, *Rhodotorula* 4.

As for the difference between the nasal cavity proper of normal persons and the diseased maxillary sinus, it was noted that *Aspergillus* was found more frequently in the latter (13%) than in the former (2%).

V. Tonsils and Fungi

The lacunae of tonsils must be safe and comfortable residence for micro-organisms. Over 20 years ago, Tanaka, F., Oda D. and others in Okayama Medical College detected that *Actinomyces* could be frequently found in tonsils. The other sorts of fungi in tonsils, however, have not ever been studied.

Examining 163 tonsils obtained by tonsillectomy, the author and his collaborator could find many sorts of fungi in about 42% of them. The fungi found and the number of each fungus were as follows: *Aspergillus versicolor* 8, *A. humicola* 6, *A. sydowii* 4, *A. sydowii* var. *columnaris* 1, *A. amstelidmi* 3, *A. proliferans* 1, *A. repens* 5, *A. ruber* 1, *A. bicolor* 3, *A. penicilloides* 1, *A. fumigatus* var. *rugophorus* 1, *A. candidus* 2, *A. flavus* 2, *A. soyae* 1, *A. sulphurens* 1, *Penicillium* 18, *Scopulariopsis* 7, *Alternaria* 5, *Stemphilium* 2, *Hormodendrum* 9, Phcmaceae 4, *Papularia* 4, *Cephalosporium* 1, *Fusarium* 1, Actinomycetaceae 4, *Mycelia sterilia* 10, *Candida albicans* 10, *C. tropicalis* 3, *C. parapsilosis* 1, *C. guilliermondii* 1, *Torulopsis glabrata* 1, *T. sake* 1, *Rhodotorula minuta* 2, *Rhodotorula* sp. 2, *Kloeckera* 1 and *Dematium* 1.

Comparing the fungi in the lacunae of tonsils with those on the lateral and posterior wall of the pharynx, it is revealed that *Aspergillus* and *Penicillium* prefer the lacunae to the pharynxwall: *Aspergillus* was found 30.7% in the lacunae and merely 1.3% on the pharynxwall, *Penicillium* 11% in the former and 2.3% in the latter. Yeasts showed, however, no difference: 13.5% in the lacunae and 14.3% on the pharynxwall.

In the examination in vitro, most strains of *Aspergillus* and *Penicillium* found in the lacunae showed to have some antagonistic faculties against *Streptococcus hemolyticus*. The cases containing fungi in their tonsils failed to show any focal infection. In the other hand, the cases with focal infections were revealed to contain no fungi in their tonsils.

Based on these findings the author would like to suggest that the fungi

ving in the lacunae of tonsils might play a significant role in preventing tonsillar focal infections. It may be similarly considered for the fungi in the diseased sinuses. Hereby, it must be mentioned that in Japan and Formosa the local infection due to chronic tonsillitis and sinusitis are found very rare than in the Western Countries. If the supposition of the author were proper, the fungi in the sinus and tonsil should be honoured as competent guards for us.

VI Thrush

Several different opinions on the causing fungi of thrush have been adopted. Some regard *Candida albicans* as the sole agent. Others mentioned several other species also, even though they believed *C. albicans* be the most essential and frequent one. On the contrary, Castellani advocated the multiplicity of thrush fungi and gave six or seven genera. He also stated that genuine *Candida albicans* was very rare in thrush.

The investigations of the author and his collaborators in Japan and Formosa verified that the genus *Candida* was the unique as the causing fungus of thrush and in the species of the genus *C. albicans* had the overwhelming predominance. They had, however, to add that some other species than *C. albicans*, particularly *C. tropicalis*, might not be neglected.

The yeasts obtained in Formosa were *Candida albicans* 131, *C. albicans* var. *croisi* 2, *C. albicans* var. *triadis* 2, *C. albicans* var. *pauchifilosa* 2, *C. tropicalis* 1, *C. guilliermondii* 4, *C. krusei* 3, *C. solani* 1 and *C. brumptii* 1; while in Japan *C. albicans* 42, *C. albicans* var. *pauchifilosa* 2 and *C. tropicalis* 2.

VII Perleche (Stomatitis angularis)

Etiology of Perleche is not yet clearly determined. Yeasts are supposed to be one of the essential factors causing the disease.

Examinations of school children revealed that 287 pupils in 4251 examined in Formosa and 218 in 10521 in Japan had the disease. The rates of the yeast positive cases were 26.5 % in Formosa and 17.4% in Japan.

The yeasts obtained in Formosa were *Torulopsis holmii* 6, *T. minor* 4, *T. tria* 3, *Candida guilliermondii* 27, *C. albicans* var. *triadis* 10, *C. albicans* 5, *C. tropicalis* 5, *C. parapsilosis* 4, *C. humicola* 4, *Rhodotorula longissima* 6 and *R. mucilaginosa* 2, while in Japan *Torulopsis sphaerica* 3, *T. stellata* 2, *T. sake* 4, *colliculosa* 1, *T. molishiana* 1, *Candida tropicalis* 11, *C. albicans* 7, *C. pelli* 2, *C. solani* 2, *C. scotii* 1, *C. formosana* 1 and *Rhodotorula mucilaginosa* 2.

In the trials to treat perleche of school children, Trichomycin ointment and 1% phenylmercuric acetate ointment showed a certain effect, while Vitamin B₂ failed to improve the disease.

VIII Deep Type Blastosporiasis in the Upper Airway

"Blastosporiasis" is a new name proposed by the author for the diseases due to yeasts and yeast-like fungi.

There has been "Blastomycosis" since Busse Buschke (1894~1895) reported a case due to *cryptococcus neoformans*, a genuine yeast.

At present, however, "blastomycosis" is usually, especially in America, applied to Gilchrist's disease due to *Blastomyces dermatidis* that is not essentially a yeast but a filamentous mold, and the disease due to *Hormodendrum* that differs far apart from yeasts is called chromo- "blastomycosis". On the other hand, thrush and other candidiasis which are caused by a true yeast, *Candida*, are not usually included in "blastomycosis".

In order to settle the universal confusion around "blastomycosis", it is likely to be adequate and also necessary to give an entirely new name there. The author's "blastosporiasis" includes cryptococcosis and candidiasis as "eublastosporiasis" and also Gilchrist's disease as well as South American Blastomycosis as "pseudoblastosporiasis".

In the upper airway there are two types of eublastosporiasis: the one is the superficial type, d. i. thrush etc, and the other is the "deep type blastosporiasis".

The deep type blastosporiasis has been appeared very rarely in medical literatures in the Western Countries. In Japan, however, it is relatively common. The author has been able to collect reports of 90 cases of the disease in Japan.

The causing yeasts in the reports were as follows: *Candida albicans* 30, *C. tropicalis* 4, *C. parapsilosis* 1, *C. krusei* 1, *Candida* sp. 10, *Cryptococcus* (or *Torulopsis*) sp. 11, *Saccharomyces* sp. 1, *Sporotrichum* sp. (?) 1, and unknown or unexamined 31.

The most remarkable finding in the disease is ulcer, usually multiple in the mouth and throat.

Site of ulcer has a wide variation: soft palate 35, tonsils 34, mesopharynx (excluding tonsils) 30, cheek 28, tongue 25, lip 21, gum 21, larynx 21, hypopharynx 19, hard palate 17, mouth angle 15, nose 12, epipharynx 9, epiglottis 9, facial pillar 3 etc.

The ulcers show pictures of much variation. They resemble frequently to aphtha, decubitus, stomatitis ulcerosa, necrotic angina, tuberculosis, syphilis or cancer. However, several characteristics can be noticed as follows: (1) extreme chronicity, sometimes over several years, (2) strong resistance against the routine therapy including many sorts of antibiotics, (3) spontaneous change of the form and site of the ulcer in relatively short time, (4) frequent co-existence of scar with ulcer, (5) inspite of marked local signs merely a slight pain is com-

plained of; swallowing is usually not hindered; mostly no fever; therefore general condition is usually good.

Diagnosis of the disease is very difficult. The rarity of the disease should partly be attributed to the difficulty of its diagnosis.

For diagnosing the disease, at first a series of similar diseases such as aphtha, decubitus, necrotic stomatopharyngitis, leucoplakia, lichen ruber planus, erythema exsudative multiforme, tuberculosis, syphilis, cancer and other neoplasms should be all excluded by clinical observations and routine laboratory examinations. Then the existence of a yeast in the diseased area must be verified. For the present purpose, repeated culturing are necessary. As culture media for detecting fungi in medicine, the author advocates the joint use of Sabouraud's glucose agar and Yano's homosulfamin medium (the details about the latter will be described further below).

Yeasts obtained must be mycologically examined and identified. Their toxicity should be also examined in animals.

Seroimmunological tests are not always useless. The author would like to recommend the intradermal test and the precipitation test, in both a polysaccharide fraction of *Candida* being employed as antigen. They are reliable to a certain extent.

For the exact diagnosis biopsy is indispensable. Though the histological findings of the disease are various, some characteristics could be noted: giant cells, microabscess, hyperplasia of the connective tissue and scar. It must be merely mentioned that the co-existence of several pictures above mentioned can be frequently found in the same case, even in the one and the same piece of specimen.

The most essential finding of all is the existence of fungi amidst the diseased area of the biopsy specimen. As staining method for the present purpose, Gram stain and Hotchkiss-McManus stain are excellent. Minami, one of my collaborators, has newly modified the latter to achieve excellent results. The Minami stain is as follows: immerse 5 minutes in 5% periodic acid; rinse in tap water; immerse in basic fuchsin sol. (basic fuchsin 0.3, 95% alcohol 15.0, aq. 285.0) 2 minutes; rinse in tap water; decoloration with 1% sodium hydrosulfite; rinse in water; stain with hematoxylin.

The treatment of the disease is more difficult than its diagnosis.

Up to the present in Japan potassium iodide, trichomycin (a new antibiotics prepared by Hosoya, S.), X-ray, insulin and *Candida*-vaccine have been applied, but any of them has failed to show any reliable and definite effect. Furthermore, the author has also tried "mycostatin" and an immune serum. Mycostatin failed to show any noticeable effect. The immune serum for *Candida albi-*

cans that had been produced in dogs in 6 months revealed to be effective: when the author used the serum on two cases that had never been improved with any drugs or any methods, the one was completely improved and the other showed a remarkable reduction of ulcers.

IX Influences of Antibiotics on Fungi and Fungus Diseases in Ear, Nose, Mouth and Throat

It is the general opinion at present that antibiotics might be one of the essential factors for the occurrence of some mycotic diseases, especially of candidiasis. The investigations and examinations carried out by the author, however, could not verify the opinion.

The number of thrush cases has not increased in spite of the wide popularization of antibiotics. Regarding the frequency of yeastpositive cases in *perlèche* and that of fungi-positive cases in sinusitis and tonsillitis, the data in 1955 show no significant difference to that in 1951, when antibiotics had not yet been so popularized in Japan.

Candidiasis of the mouth and throat alone has recently become relatively common and the case reports of the disease appear more frequently in medical journals of Japan than in the pre-antibiotics era. The majority cases, however, had not the history that a large quantity of antibiotics was administered before the occurrence of the disease.

X Culturing and Identification of Fungi having Redations to Otolaryngology. New Species and New Varieties found

For detecting fungi in ear, nose, mouth and throat, the author recommends the joint use of Sabouraud's glucose agar and Yano's homosulfamin medium. The former gives rich growth of fungi, but at the same time it gives some growth of bacteria and the bacterial colonies frequently disturbs the matter. The latter, Yano's medium, usually prohibits any growth of bacteria and it gives excellent results than Littman's medium. Though the Littman's medium may begin to give some bacterial growth about one month after its preparation, due to a decreasing of the bacteriostatic power of streptomycin added to it, the Yano's medium does not lose the ability to suppress the growth of bacteria even after one month. Yano, one of the author's collaborators, found the medium the best among reknowned media as well as many newly media devised by himself. It consists of potato-decoction 100, potassium phosphate monobasic 0.2, potassium phosphate dibasic 0.07, asparagine 0.3, ammonium sulfate 0.2, glucose 3.0, homosulfamine 4.0 and agar 2.0.

Though many studies on identification of various fungi in otolaryngological

urea have been done, in this abstracted report mere that of *Candida* will be described.

Before the identification of *Candida* species, it is necessary in exact studies to purify the strain from bacteria by acid Raulin and also to prepare a strict pure strain by the single spore culture method, because the author's experiments have revealed that without passing through the procedures above mentioned a strain might give some different results in the fermentation test and other tests.

The occurrence of the difference is, however, relatively rare and the grade of the difference is usually negligible. Therefore, the acid-Raulin and the single spore culture might be omitted in clinical laboratories, where it is more desirable to get quickly some fair findings than to get ones of complete exactness in long time.

From the standpoint of mycology, tests of ascospore formation are indispensable for identifying yeasts. The author and his collaborators have endeavored to get ascospores in all the strains obtained from ear, nose, mouth and throat. In first place the strains were cultured on Lindegren's pre-sporulation media for 3~4 days and then transferred to Gorodokowa, Carrot, Gips, Koji-agar and McKelvey's carrot-gips media. The cultures were examined once a week till the fifth week, using Hantsch's solution, double Lugol solution and Kuffenhath's stain modified by Langeron, and if something similar to asci or ascospores were found, they were also examined by the Suminoe's germination test, a test to assure them to be ascospores by causing germination or sprouting.

In our examinations only 7 of all 671 yeast strains could produce ascospores. As the ascosporogeneous yeasts are thus very rare in medical area, tests for ascospore formation that take much time and labor and are very difficult for the beginners may be omitted in the routine work of the clinical laboratories.

The formation of pseudomycelium must be always examined, if one wish to identify a yeast-like fungi. The author's experiments revealed that the slide culture using potato-carrot agar was the best for that purpose. The cultures in liquid media and those in petri dish are inferior to the slide culture. The potato-carrot agar is superior to any of Sabouraud agar, corn meal agar and others in examining pseudomycelium.

It seems to be generally accepted by medical mycologists that the *Candida* species with Chlamidospores are always *C. albicans*, and that all other *Candida* species did not produce them. That is true in general but not always so. A *Candida* species, *C. formosana*, obtained by the author from otomycosis in For-

mosa showed typical chlamidospores just like those of *C. albicans*, though it was much different from *C. albicans* in sugar fermentation and many other qualities. This exception is, however, very rare: only 2 of about 500 *Candida* strains examined by the author. Therefore, in clinical laboratories, the *Candida* species with chlamidospores may be considered as *C. albicans*.

The best method of investigation for chlamidospore formation might be, after the author's experiments, the slide culture utilizing three media jointly, potato-carrot agar, Nickerson's starch-trypan blue agar modified by Donomae, I. and corn meal agar. By the present method the author verified chlamidospores in 95 of 100 *Candida albicans* strains.

Though several trials have been carried out to classify *Candida* species by serological methods, they are likely to have no practical value. Recently Tsuchiya, T., Tokyo, has devised a new serological method that is, according to his descriptions, very reliable and practical. Idei, K., one of the author's colleagues, has also studied the problem and succeeded in producing the sera for identifying *Candida* species. His sera were prepared by injecting seven different *Candida* species intravenously in rabbits and by absorbing some antibodies from the sera with corresponding antigens.

Table 7

Strains tested	No of Serum							
	1	2	3	4	5	6	7	8
<i>C. albicans</i>	+	-	-	-	-	-	++	-
<i>C. tropicalis</i>	++	+	-	-	-	-	++	-
<i>C. pseudotropicalis</i>	-	-	++	-	-	-	-	++
<i>C. krusei</i>	-	-	-	++	-	-	-	-
<i>C. parapsilosis</i>	-	-	-	-	++	+	-	-
<i>C. guilliermondii</i>	-	-	-	-	+	-	-	-
<i>C. stellatoidea</i>	-	-	-	-	-	-	++	++

Agglutinations of the 7 *Candida* strains used for producing the sera by each of the sera were as shown in Table 7. Based on this datum, it might be assumed that the seven species could usually be identified with these sera.

To know whether this assumption is proper or not, many other *Candida* strains other than the concerned seven were examined with the present sera. The result was that this method could give the consistency with the mycological identification in about 83%. The author fancies that the serological method for identifying *Candida* species would make much progress and might become more reliable in the future, when *Candida* will be readily identified by

any technician in clinical laboratories.

New species and new varieties and established by the author in Japan and Formosa during about 20 years are as follows;

1. *Lichtheimia maligna* n. sp.
2. *Aspergillus fumigatus* var. *formosanus* n. var.
3. *Aspergillus fumigatus* var. *major* n. var.
4. *Aspergillus fumigatus* var. *rugophorus* n. var.
5. *Aspergillus terreus* var. *albidus* n. var.
6. *Aspergillus terreus* var. *pauper* n. var.
7. *Aspergillus terreus* var. *pallidus* n. var.
8. *Aspergillus sydowii* var. *microvesicularis* n. var.
9. *Aspergillus sydowii* var. *procerus* n. var.
10. *Aspergillus sydowii* var. *rugophorus* n. var.
11. *Aspergillus sydowii* var. *columnaris* var.
12. *Aspergillus versicolor* var. *rugophorus* n. var.
13. *Aspergillus versicolor* var. *thermophilus* n. var.
14. *Aspergillus paranidulans* n. sp.
15. *Scopulariopsis blochii* var. *alba* n. sp.
16. *Scopulariopsis nivalis* n. sp.
17. *Scopulariopsis humilis* n. sp.
18. *Scopulariopsis fuliginosa* n. sp.
19. *Scopulariopsis divaricata* n. sp.
20. *Candida taiwana* n. sp.
21. *Candida taiwana* var. *catenata* n. var.
22. *Candida otica* n. sp.
23. *Candida otica* var. *pulvenaris* n. var.
24. *Candida otica* var. *petasata* n. var.
25. *Candida formosana* n. sp.
26. *Candida albicans* var. *sucrosa* n. var.
27. *Candida albicans* var. *pauchifilosa*

The detailed descriptions of most of the new fungi above mentioned have been reported in Japanese in "Nittai Igaku" (Tropical Medicine, Formosa) and 'Jibiinkoka Rinsyo' (Otorhinolaryngological Clinic [Kyoto]), and those in English will be published in the near future.