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CONCERNING A FUNGUS (ATELOSACCHAROMYCES CATANEI) ISOLATED FROM A PATIENT WHO DIED OF ILL-DEFINED INFECTION

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Introduction

Formerly clinical studies on the fungous diseases of man were prosecuted only in the dermatological field. But recently owing to the wide use of antibiotics such as penicillin, streptomycin, aureomycin, chloromycetin, terramycin etc., and like so many blots on the brilliant therapeutic reputation which these antibiotics have achieved by fighting down common bacterial infections, a number of papers dealing with the fungous diseases which appeared during or after treatments with these antibiotics have come to be published (1, 2, 3, 4, 5); and now in every branch of medicine a deep interest in fungous diseases has been aroused. If we review these papers, however, we find that almost all the cases reported are those of moniliasis, namely, mycosis caused by Candida albicans, and so we have a false impression that moniliasis is a single fungous disease associated with the use of antibiotics.

Some time ago a patient died of an ill-defined disease with a continual high fever in the Kobe Medical College Hospital. In the urine, sputum and so on of this patient we detected a fungus of a certain kind in great and constant numbers, and we were able not only to isolate and culture this fungus, but also to demonstrate by biologic and serologic examinations that it was an yeastlike microorganism quite distinct in various characters from every species of Candida. Furthermore, we tested the complement fixation test of the patient's serum against this fungus, traced the fungus in her viscera and conducted other examinations in order to establish almost conclusively that the cause of her death was due to infection with this isolated fungus. In this paper, of all the clinical and laboratory examinations done concerning this patient, the mycologic one shall be chiefiy described.

Bacteriologic Examination

A patient with unsettled diagnosis who presented clinical aspects of septicaemia was referred to us by the medical clinic of the Kobe Medical College Hospital for the detection of etiologic agents. And so the following examinations were done.

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(1) Culture of the patient's blood on the bile medium for the organisms of the Salmonella group, and animal inoculation of the same.

(2) Staining of the sputum by Gram's and Ziehl-Neelsen's methods, and culture of the same for the tubercle bacillus.

(3) Staining by Gram's method of the urine sediments, and culture on Endo's medium of the above material and feces for the enteric bacilli.

(4) The Widal test of the serum.

All these tests gave negative results for the tubercle and enteric bacilli; as for the Widal test, agglutination was at 1 : 20 for the typhoid bacillus, and for the Salmonella paratyphi A and B at 1 : 10. However, a large number of yeastlike microorganism were always found in the smear preparations of urine, sputum, feces and pharyngeal swabs at repeated examinations; and not only that, but these organisms were present in pure-culturelike abundance in the urine aseptically collected.

Selective Cultures of Yeastlike Organisms

For the purpose of culturing these yeastlike organisms in a selective way, Sabouraud's glucose agar was inoculated with materials from urine, sputum, feces and pharyngeal swabs, but this attempt failing, especially with feces and pharyngeal swabs on account of admixture of many saprophytes, we were induced to use Littman's OSCV medium devised for the selective culture of fungi. (6, 7) (cf. Table I). On this medium after incubation of 24-48 hours at 37°C or

TABLE I

Littman's OSCV medium

peptone	10 gm.
dextrose	10 gm.
powdered beef bile	15 gm.
crystal violet	9.2 c.c.
streptomycin	30γ/c.c.
water	1000 c.c.

Notes; 1) The reaction is not adjusted.
2) Crystal violet solution is prepared by solving 1.25 gm. of crystal violet in 95% alcohol.
3) Streptomycin is added when the medium has cooled down to about 45°C

room temperature the isolated fungus formed a colony, 1—2 mm. in diameter; this colony was white or slightly purplish, had a flat, moist surface and creamlike consistency, and gave out a sort of sweet smell.

Biologic Properties

Hitherto many methods have been tried for the differentiation, identification and classification of fungi; Castellani emphasized differential fermentations (8), Langeron attached importance to morphology, especially to that of mycelium, and Benham attempted the classification from the biologic and serelogic viewpoints (9). All these methods, though excellent in their own way, are still far from being infallible. Moreover, what the clinician needs is a practical, simple as well as sure method of identification. Recently Martin et al. (10, 11) have introduced a new method which satisfies this clinician's need much better than the above-mentioned ones. This method includes observations on the following points.

(1) Pellicle formation and gas production on Sabouraud's broth.
(2) Morphology of adult colony on blood agar.
(3) Mycelium and chlamydospore formation on corn meal agar.
(4) Sexual spore formation on sporulation medium.
(5) Sugar fermentations on a variety of sugar broths.

It must be admitted, however, that though this method is quite effective in the differentiation of the species of Candida, it is not sufficient for the differentiation of other fungi. Therefore, for the identification of our isolated fungus we added other examinations to Martin's method. All the biologic examinations we conducted are tabulated in Table II.

TABLE II

Biologic examinations for differentiation and identification.

Culture media	Chief points observed
1. Sabouraud's agar 2. Sabouraud's conservation medium 3. Coagulated serum 4. Gelatin 5. Carrot 6. Potato chips	(1) Fungous growth (2) Colonial morphology (3) Fungous size (4) Gelatin liquefaction
Sabouraud's broth	(1) Pellicle formation (2) Gas production
Blood agar	Morphology of adult colony
1. Corn meal agar 2. Potato agar	(1) Pseudomycelium formation (2) Chlamydospore formation
1. Carrot 2. Gorodkowa's medium 3. Gypsum	Sexual spore formation
1% Sugar-Peptone broth	Sugar fermentation
1% and 10% Sugar agar	Sugar utilization
Milk	Rennet curd precipitation

In addition to the examinations enumerated in Table II the biologic characters of our isolated fungus were compared with those of Candida albicans FIA1001 and Candida parakrusei, which we had got by the courtesy of the Osaka University Institute for Microbiology.

(1) Size and Morphology of the Isolated Fungus Cell.

The fungus cell on Sabouraud's glucose agar was 2—3μ in diameter; spherical or ovoid; in a yeastlike form; budded forth in the direction of its longer axis; and in unstained preparations was seen as having a few granules in its cytoplasm. And the cytoplasm of the younger cells, it is to be added, was dotted with oily droplets, and that of the older ones with big vacuoles. This fungous cell was further observed as having a very resistant cellular membrane, the toughness of which was proved by the fact that it remained undamaged after being repeatedly subjected to the freezing and melting process. Candida albicans and Candida parakrusei, on the other hand, were rather larger than this fungus in question, being 3—6μ in diameter, and spherical or ovoid in shape. In their cytoplasm neither oily droplets nor vacuoles were present, and they were easliy broken by application of the freezing and melting process.

(2) Growth, Colonial Morphology and Size of the Fungus Cell on a Variety of Culture Media.

Colonies of this isolated fungus on potato were yellowish white and rather dry; on carrot very small, moist, white and spherical; on Sabouraud's agar moist, white and spherical; on Sabouraud's conservation agar showed poor growth; on coagulated serum no growth; on gelatin feeble growth along line of stab and caused no liquefaction. Practically no difference was noted in cell size on these various media, except on Sabouraud's conservation agar on which the cell looked rather thinner and longer. As for Candida albicans and Candida parakrusei, they showed more active growth on these media than the fungus in question, growing even on serum, but they, too, did not liquefy gelatin.

(3) Sabouraud's Broth.

The isolated fungus on Sabouraud's broth produced neither gas nor pellicle 48 hours after inoculation, and so was it with Candida albicans and Candida parakrusei. Sediments, however, were rather finer with the isolated fungus than with the organisms of Candida.

(4) Adult Colonies on Blood Agar.

The method of adult colony observation was first described by Martin et al. In the present investigation their method was followed.

Adult colonies of the isolated fungus on blood agar were less than 1 mm. in diameter, white, moist, sheeny and spherical with a smooth rim. Those of Candida albicans were about 2 mm. in diameter, greyish white or white, moist, sheeny and spherical; those of Candida parakrusei less than 1 mm. in diameter, white, sheeny and spherical.

(5) Pseudomycelium and Chlamydospore Formation on Corn Meal and Potato Agar.

Formation of pseudomycelia and chlamydospores is a decisive point in the differentiation of fungi. For the investigation of this point such media as corn meal and potato agar are used (9, 12, 13), and as methods of inoculation there are cut-streak and slide-culture methods (9).

Being inoculated on the above-mentioned media by the above-mentioned methods, Candida albicans and Candida parakrusei were observed actively producing pseudomycelia 48—72 hours after inoculation ; and especially in the former, from the sixth day after inoculation onward, was noted the formation, singly or several cells clinging together, of chlamydospores at the ends of arborescently formed pseudomycelia. On the contrary, pseudomycelium formation in the isolated fungus was not disclosed by a three-week long observation, not to mention of chlamydospore formation, though the culture was repeated several times. By this fact the fungus in question was undeniably shown to be not of the species of Candida.

(6) Sexual Spore Formation

To ascertain the existence or not of the sexual phase in the isolated fungus we incubated it on gypsum, carrot and potato chips media for two weeks, and on Gorodkowa's medium for five weeks. Unstained preparations were chiefly used for the demonstration of sexual spores, but to remove the errors by overlook as much as possible Ziehl-Neelsen's and Moeller's staining methods were also made use of. By the way, by these methods sexual spores stain red. The fungus in question being found as asexual like the organisms of Candida, it was classed with the Fungi Imperfecti.

(7) Sugar Fermentations

As methods to examine sugar fermentations, Lindner's, Einhorn's, Durham's, Guerra's and other methods are in current use. But owing to both its simpleness in manipulation and its ability to demonstrate a tiniest amount of gas produced, Guerra's method is most popular in European and American laboratories. In the present investigation on this point modified Guerra's method was employed (14). That is, sugar-peptone solution was tubed, boiled for several minutes to expel air from tubes, and then, after being cooled, inoculated with a certain amount of fungous suspensions. In order securely to collect gas, the material consisting of equal parts of veseline and paraffin was poured into the tubes to a height of 4—5 mm. above the surface of the culture.

These media were left at room temperature or 37°C for 21 days and observed for gas production. The isolated fungus produced gas and acid on glucose and levulose, but exerted no action on other sugars ; Candida albicans produced both gas and acid on glucose, maltose and levulose, but acid only on sucrose and galactose ; and Candida parakrusei, like the fungs in question, produced gas and acid on glucose and levulose. On milk each of these fungi developed no rennet curd precipitation.

(8) Sugar Utilization Test.

It is Langeron and Guerra (15) who first applied this test, which is now

widely used in the zymologic studies, to the group of asporogenous yeasts. In the present investigation this test was done after Beijerinck's method. That is, agar media inoculated with fungi were spread with sugar solutions of certain concentrations, and observed for fungous growth. If fungous growth took place, it was regarded as a positive result. But agar often happens to contain sugars, and when the agar of this kind makes a constituent of the media, fungi grow as a matter of course even on the media not spread with sugar solutions, making the test meaningless. Therefore, the agar used in this test was washed beforehand in a running water, and sugars were removed from it. 0.1, 1.0 and 10.0% sugar solutions were used as spreading solutions. But in case 0.1% solutions were spread, fungous growth was generally so poor that judgement of results became difficult. Accordingly, in the present test, results were taken from only the media spread with 1.0 and 10.0% sugar solutions.

Of course we had previously made sure of no growth on the control media not spread with sugar solutions. Growth of the isolated fungus was noted on glucose, levulose and galactose ; as for Candida albicans, it gave a positive result on glucose, levulose, galactose, maltose and sucrose ; and in the case of Candida parakrusei, growth was possible only on glucose and levulose.

* * * * *

The biologic properties of the isolated fungus, Candida albicans and Candida parakrusei, as revealed in the above examinations, are presented in a comparing way in Table III.

As shown in Table III, the isolated fungus has much in common with common yeasts in biologic properties, but unlike common yeasts, it has no sexual phase, and so it is classifiable in the Fungi Imperfecti as one of the asporogenous yeasts. This fungus is also different from the species of Candida in that it produces no mycelium and reproduces itself by budding. Many investigators have already made extensive studies concerning the asporogenous yeasts, and advanced a variety of taxonomical methods, but still any of these methods is far from being perfect. Under such circumstances it is natural that many kinds of these fungi should have a lot of different names given by different nomenclators. Skinner (16) classified the asporogenous yeasts in the following manner :

(1) Sporobolomycetaceae
(2) Rodotorulaceae
(3) Cryptococcaceae
  (a) Cryptococcoideae
  (b) Candidoideae

Some members Sporobolomycetaceae produce no pseudomycelium, but sugar fermentations are unknown to every members of the groups. Rodotorulaceae's characteristic is pigment production. Cryptococaceae of course produce no pigments, and propagate by budding, but they differ among themselves on pseudo-

TABLE III

Biologic Characteristics

Fungi		Isolated Fungus	Candida albicans	Candida parakrusei
Fungus growth & Colonial morphology	Sabouraud's agar	2–3 mm. in diameter, white, moist, spherical	2–3 mm. in diameter, white, moist, spherical	2–3 mm. in diameter, white, moist, spherical
	Sabouraud's conservation medium	poor growth	better growth	better growth
	Coagulated serum	no growth	growth	growth noted
	Carrot	small, white	yellowish white, active growth	yellowish white, active growth
	Potato chips	yellowish white, active growth	yellowish white, active growth	yellowish whire, active growth
	Sabouraud's broth	pellicle —	—	—
		gas —	—	—
	Blood agar (Adult colony)	less than 1 mm. in diameter, white spherical	2–3 mm. in diameter, greyish whire, spherical	less than 1 mm. in diameter, white, spherical
Pseudomycelium		−	+	+
Chlamydospore		−	+	−
Sexual spore		−	−	−
Sugar fermentation	Sucrose	−	A	−
	Glucose	AG	AG	AG
	Maltose	−	AG	−
	Mannitol	−	−	−
	Galactose	−	A	−
	Xylose	−	−	−
	Arabinose	−	−	−
	Lactose	−	−	−
	Levulose	AG	AG	AG
	Raffinose	−	−	−
Sugar utilization	Glucose	+	+	+
	Levulose	+	+	+
	Galactose	+	+	−
	Maltose	−	+	−
	Sucrose	−	+	−
	Lactose	−	−	−
Gelatin liquefection		−	−	−
Development of rennet curd on milk		−	−	−

Notes: In sugar fermentation A is for acid production; G is for gas production.

mycelium formation and sugar fermentations: pseudomycelium formation is generally alien to one type of this group, Cryptococcoideae, but takes place

actively in another type, Candidoideae. According Skinner's classification, therefore, the fungus in question may be looked upon as a variety of Cryptococcoideae.

Saito's classification (17) of the asporogenous yeasts runs as follows :

(1) Hyalotorula
  (A) Kleockeria
  (B) Torulopsis
    (a) Eutorulopsis
    (b) Torulopsis (in a narrow sense)
(2) Mycotorulae
  (A) (a) Mycotorula
    (b) Mycoderma
  (A) (a) Pseudomycoderma
    (b) Pseudomonilia

According to Saito's classification the isolated fungus belongs to the group of Eutorulopsis, its biologic properties coinciding accurately with those of Eutorulopsis Hansen. The name, 'Eutorulopsis Hansen' comes, by the way, from zymologic nomenclature.

According to Dodge's Medical Mycology (18, 19) which gives detailed descriptions of pathogenic fungi, the isolated fungus tallies in biologic properties with Atelosaccharomyces Catanei. It may not be out of place here to add that though Dodge says that Atelosacharomyces Catanei forms very small colonies on carrot, we noticed in the present investigation that colonial morphology of the isolated fungus was dependent upon the nature of carrot used : different results were got, if different kinds of carrot were used, and only when the so-called European carrot was used, our results agreed with Dodge's description.

Quest for the Fungs in Question in the Viscera

We strove to prove the presence of the fungus in question in the patient's viscera by staining and cultural methods in order to establish the pathogenicity of the said fungus for her, and to attest that her death had been due to infection with the said fungus, and that this infection had been a systemic one, the clinical aspects of which she had clearly presented in life.

Tissue sections were prepared from the internal organs fixed in Zenker's solution, stained in Goodpasture's stain (20), and then examined. Tissue sections of lung, kidney, spleen and tongue were found to contain gram-positive, spherical or avoid yeastlike microorganisms, 2—3 mm. in diameter. And these organisms were without capsule such as that of Cryptococcus neoformans. In sections stained in Goodpasture's stain, however, particles of hemosiderin present a form and colour much resembling those of fungi. Therefore, to eliminate the error of confusing fungi with hemosiderin, sections were again stained in Berlin blue. Thus stained, ascanty amount of hemosiderin was detected, it is true, but at

the same time were demonstrated a good many gram-positive yeastlike bodies which gave a negative iron reaction. We thought we were safe in assuming these bodies as fungi.

Culture of the fungus in question was done with heart blood and with lung, liver, spleen and kidney tissues. Organ tissues were emulsified and diluted 1:10 with physiological saline solution. The suspensions thus prepared were then spread on Sabouraud's media, which were kept for 10 days at 37°C, but no fungous growth whatever was noted. This negative result may be due, as Fujino pointed out, to our neglecting to inhibit saprophytic growth by soaking the organs in penicillin solution before culture.

Pathogenicity of the Isolated Fungus for Animals

Pathogenicity of the isolated fungus for rabbits and mice was next investigated. Rabbits were intravenously infected in 10 mg. quantities with the fungus, and mice intraperitoneally in 2 mg. quantities. As nothing unusual was found with rabbits during one-month-long observation period after infection, the fungus in question may be looked upon as being non-pathogenic for rabbits.

Three mice inoculated with the fungus taken from the patient just before inoculation erected their fur and became restless from about the 14th hour after inoculation on, and all died 36—48 hours later. Immediately after their death an attempt was made to culture the fungus from heart blood and internal organ tissues. Many fungous colonies formed on the media inoculated with heart blood, and kidney and spleen tissues. Of three mice inoculated with the fungus which had gone through several serial transfers on artificial media, one mouse perished, and the surviving two were sacrificed two weeks after inoculation, and we succeeded in culturing the fungus in a great number from kidney and spleen tissues. It was also noted that attenuated fungous virulence resulting from repeated serial transfers on artificial media could be restored back to its former titre by passing the fungus two or three times through mice. Though abscess formation is the rule in Candida albicans infection, no such lesions were macroscopically detectable in the viscera of these mice. In tissue sections of spleen and kidney stained in Goodpasture's stain the fungus was demonstrated in a great number.

Serologic Properties

In order to get more concrete evidence of pathogenicity of the isolated fungus the complement fixation tests of the patient's serum against this fungus in question, Candida albicans and Candida parakrusei were investigated. In fungous infection, as tests of immunologic reaction, are used the same procedures as in the case of common bacterial infections, namely, agglutination, precipitation, complement fixation and so on. We first resorted to agglutination

test but as this test very often caused a spontaneous agglutination in the present case, we discarded it for complement fixation test. The antigens were prepared in the following way : the suspensions which contained 10 mg. of the fungi per 1 cc. of physiologic saline solution were heated at 60°C for 30 minutes, shaken for 5 hours, and then centrifugalized, the resulting supernatant being used as antigens. The test was done after the Kolmer technic, and the sera of fifteen healthy persons were used as controls. The reactions of the respective sera of the patient and fifteen controls against the isolated fungus, Candida albicans and Candida parakrusei are presented in Table IV.

TABLE IV

Results of Complement Fixation Test

Sera \ Antigens		Isolated Fungs	Candida albicans	Candida parakrusei
The Patient		×20(+)	×10(+)	×10(−)
Healthy Persons		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)
		×10(−)	×10(−)	×10(−)

As shown in the table, the patient's serum agglutinated the isolated fungus at 1:20, and Candida albicans at 1:10. The reaction was negative for all controls.

Next cross complement fixation test was carried out with the antisera for the isolated fungus, Candida albicans and Candida parakrusei. Heated saline suspensions of these fungi were used as vaccines, but as the antisera got by this means were all of low titre, this test proved a failure. Therefore, we used as vaccines suspensions of these fungi repeatedly treated by the freezing and

melting process. These vaccinal suspensions were prepared as follows : suspensions which contained 10 mg. of the fungi per 1.0 cc. of saline solution were frozen, by being put in the admixture of aceton and dry ice, and then melted in warm water of 37°C. Stained smear preparations were made from these frozen and then melted suspensions to examine whether the fungi were damaged or not by this process. This process was repeated till the fungi got broken. Candida albicans and Candida parakrusei withstood this process four times, and the isolated fungus seven times. Suspensions of the fungi thus treated were diluted 1:10, and 0.7, 1.0 and 1.5 cc. of them were injected, three times at an interval of five days, into the auricular veins of white rabbits which weighed 2—2.5 kg. On the 7th day after the last injection the blood was taken from these rabbits, and the separated serum was stored away in a refrigerator. The antigens were prepared, as already mentioned, from the supernatant of centrifugalized fungous suspensions. The results of this cross complement fixation test concerning these three kinds of fungi are presented in Table V.

TABLE V

Results of Cross Complement Fixation Test

Antisera \ Antigens	Isolated fungus	Candida albicans	Candida parakrusei
Isolated Fungus	×40(+)	×10(+)	×10(+)
Candida albicans	×10(+)	×40(+)	×10(−)
Candida patakrusei	×10(−)	×10(−)	×40(+)

As shown in the table, the antiserum for the isolated fungus agglutinated this fungus at 1:40, and Candida albicans and Candida parakrusei at 1:10 ; the antiserum for Candida albicans reacted with the isolated fungus at 1:10, and with Candida albicans at 1:40, but its reaction against Candida parakrusei was negative at 1:10 ; and as for the antiserum for Candida parakrusei, its agglutinations of the isolated fungus and Candida albicans did not occur at 1:10, but it specifically reacted with Candida parakrusei at 1:40. Thus the respective specificities of these antisera were clearly recognized.

Attempts at Isolation of the Fungus in Question from Healthy and Sick Persons

Our attempts to isolate the fungus in question from the urine, pharyngeal mucus and so on of fifteen healthy persons by the same methods as described above witnessed a complete failure. About thirty patients referred to us by the medical and the ear, nose and throat clinics for mycologic examination were also examined for the same purpose, but no fungi which might be taken as the same

with the fungus in question could not be detected. From this, this fungus, unlike Candida albicans, does not seem to be widely distributed in nature as non-pathogenic organisms.

Summary and Discussion

We isolated a certain kind of yeastlike microorganism from a patient who died of ill-defined disease, and, subjecting this organism to biologic and serologic examinations, found that it coincided with Atelosaccharomyces Catanei in Dodge's Medical Mycology, and with Eutorulopsis Hansen according to Saito's description. And it was carefully investigated whether this organism merely maintained a parasitic mode of existence in the patient as a saprophyte, or was the cause of the patient's fatal disease. Fungi, as a general rule, are observable in healthy persons and patients suffering from other diseases than mycoses; Candida albicans, for instance, is said to be recoverable from 10—25% of healthy persons (21, 22, 23). Moreover, as the fungi recovered from healthy persons and those from lesions have the same biologic properties, it is impossible at present to differentiate them from each other. And so, to settle the diagnosis of mycosis, we must see, just as in the case of common bacterial infections, that a given fungus presumed as etiologic agent fulfills Koch's postulates concerning etiologic relationship. For this purpose, in the present case, the constant demonstability in the patient, and non-demonstrability in healthy persons, of the fungus in question were first investigated; secondly the complement fixation test of the patient's serum against the said fungus; and lastly pathogenicity of the said fungus for mice, and pathologic changes in infected animals. Results of these investigations are as follows:

(1) The said fungus was found in the urine, sputum, pharyngeal swab and so on of the patient at every examination repeatedly conducted while it was demonstrable in none of the materials take from many healthy persons.

(2) The patient's serum was found to have specific complement-fixing antibodies for the said fungus. Smith stated elsewhere (24) that complement-fixing antibodies for fungi are present in serum only in advanced cases of mycoses, but not in light cases and when fungi exist in the body as non-pathogenic saprophytes. Therefore, in view of the proved presence in the serum of complement-fixing antibodies for the said fungus, this fungus may safely be assumed as responsible for the patient's fatal disease.

(3) Though culture of the said fungus from the viscera failed because of the already-stated reason, fungous invasion into the vital internal organs was clearly demonstrated in stained tissue sections. This fact shows that the patient was a case of systemic fungous infection.

(4) The isolated fungus had no pathogenicity for rabbits, but it was plainly pathogenic for mice. Dodge says that Atelosaccharomyces Catanei is not pathogenic for rabbits and guinea pigs, but he gives no mention of its pathogenicity

for mice. The isolated fungus and Atelosaccharmyces Catanei are the same in that they are not pathogenic for rabbits.

As above seen, the isolated fungus fulfills Koch's postulates, and if results of other bacteriologic examinations are taken into consideration together with this fact, it may be a safe conclusion that the patient was a case of systemic infection with this isolated fungus.

Catane's report on his isolation of Cryptococcus sp. Catanei from cases of black pilose tongue is at present the only one report concerning the disease caused by Atelosaccharomyces Catanei, and of course no case of systemic infection with the said fungus such as was just described above is on record in the whole medical literature.

The cause of this patient's systemic fungous infection was left unexplored, as we had only a brief contact with her when she was alive, but it is a noteworthy fact that he was administered a large amount of antibiotics. Up to now several theories (25, 26) have been put forward concerning the causes of fungous infections, but the common feature of all these theories is the attribution of their main cause to the large administration of antibiotics. In the present case, too, the large administration of antibiotics seem to have hastened the fatal course of the disease.

Concerning bacteria, the systematic taxonomy, as represented by Bergey's Manual of Determinative Bacteriology, is already established on a firm basis. But in the realm of fungi taxonomy and nomenclature are still in infant stage, and so, different investigators give different names to the same and one fungus: Candida albicans, for example, has one hundred and seventy-two synonyms. This confusion in fungous taxonomy is the source of constant embarrasment to the students of mycology. In biologic properties our isolated fungus agrees with Eutorulopsis Hansen according to Saito's classification, and with Atelosaccharomyces Catanei according to Dodge's one. We wanted to ascertain whether or not our isolated fungus belongs to the same species as Eutorulopsis Hansen and Atelosaccharomyces Catanei, and whether Eutorulopsis is really the same with Atelosaccharomyces. But though we did all in our power, we could not find a possessor of these fungi. Careful comparison of biologic properties of our isolated fungus with those of Saito's and Dodge's fungi, however, leads us to think that these three fungi are in reality the same fungus differently named. Saito's name, 'Eutorulopsis Hansen' seems to have been given from the standpoint of zymologic industry while Dodge's one, 'Atelosaccharomyces Catanei' from that of medical mycology.

Conclusions

An yeastlike microorganism was isolated from a patient who died of ill-defined infection, and its biologic properties were examined together with its complement fixation test with the patient's serum. It was further tried to

establish its presence in the viscera. Its pathogenicity for mice was also studied. And the following results were got.

(1) The isolated fungus agreed with Saito's Eutorulopsis Hansen, and Dodge's Atelosaccharomyces Catanei.

(2) Specific complement-fixing antibodies for the isolated fungus were demonstrated in the patient's serum.

(3) The said fungus was detected in a great number in stained tissue sections of kidney, lung, spleen and tongue.

(4) The said fungus was pathogenic for mice.

(5) In view of the above findings, it may be concluded that this patient was a case of systemic infection with the said fungus.

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