A MICROBIOLOGICAL STUDY OF TORULOSIS

By

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TABLE OF CONTENTS

LIST OF TABLES

SECTION I

SCOPE OF THE PROBLEM

Torulosis is uniformly fatal when the central nervous system is involved. The object of this research is to determine the nutritional requirements of *Torula histolytica* when cultivated in a chemically defined medium in hope that such information may lead to a more adequate treatment for this and allied mycotic infections. At the same time, the study involves the use of chemical compounds to combat the causative agent both *in vitro* and *in vivo*.

SECTION II

INTRODUCTION

Clinical Record. The fungus responsible for torulosis in man was called *Torula histolytica* by Stoddard and Cutler (1916). At the present time the organism is given the name *Cryptococcus neoformans* or *Cryptococcus hominis*. *Torula meningitis* isolated from the meninges and from lesions simulating brain tumor or brain abscess, *Torula Plimmer* isolated by Weiss (1902) from cancer of the breast and *Torula Sanfelice* from an adenocarcinoma of a human ovary are now considered to be identical. There have been many early reports concerned with budding yeast-like fungi. As far as we have been able to ascertain, the first case in which the central nervous system was implicated was reported by Zenker in 1861. At the same time the exact nature of the

causative organism was not determined; possibly it was Torula histolytica. Subsequently Busse (1894) reported another case of a woman who suffered from chronic enlargement of the lymph glands. She later developed a localized subperiosteal infection of the tibia and finally died with multiple lesions of the skin and viscera. During life, and later at autopsy, an encapsulated yeast-like fungus was cultivated. It was injected by Buschke into the patient's skin, and was found to reproduce the characteristic lesion.

Gilchrist in 1896 described a yeast-like organism in a lesion of a hand; and two years later, together with Stokes, recovered another strain of the same organism from a man with multiple cutaneous lesions.

Curtis in 1896 described the case of a young man who developed swellings of the lumbar region. The appearance of the swelling suggested to him the presence of a myxosarcoma. However, on microscopical examination, organisms were identified which when cultivated were found to be pathogenic to experimental animals.

In 1902, Frothingham described a Torula species isolated from a tumor mass in the lung of a horse. In Germany, von Hansemann (1906) isolated a yeast by lumbar puncture from a case of suspected tuberculous meningitis; and two years later Türk (1907) of Vienna reported a case of yeast infection from which he succeeded in cultivating the organism. Brewer and Wood in 1908 described a patient with a deep abscess of the back. From this case Zinsser was successful

in isolating an organism which was definitely Torula histolytica.

Verse (1914) found in a twenty-nine year-old woman a widespread leptomeningitis of the brain and spinal cord. On histological examination of these tissues, he noticed cellular infiltration with large phagocytic cells containing yeast cells showing no sporulation and he regarded it as a kind of Torula. This is the first human case correctly diagnosed as torulosis.

In this country torular infections were the subject of an excellent monograph by Stoddard and Cutler (1916). They studied two cases from Cushing's clinic, and by comparative study with animal inoculation, they came to the conclusion that the two cases were torular infection. At one time it was thought torulosis in America was confined to the San Joaquin Valley in California, but now it is known to be of wider distribution. Only six cases have been reported in Great Britain (Smith and Crawford 1930, Greenfield et al. 1938, Blair and Magarey 1943, Denton 1948, Daniel 1949, and Schiller and Vollum 1949). In Australia, Cox and Tolhurst (1946) collected and described thirteen cases in 10 years.

A diffuse chronic meningo-encephalitis is the commonest manifestation of the infection. Over 120 cases of torulosis have been reported in the literature. Probably a certain number of these cases were tuberculous meningitis.

Epidemiology. Torula organisms are spherical, yeast-

like fungi which reproduce by budding and occur in many foodstuffs. The strychnine-like bitterness noted in condensed milk in opened cans is attributed to a lower yeast, a torula, carried in stormy periods by gnates (Guyot 1945). The drops of dried milk at the orifices of a punctured can are thus contaminated. This bitterness is noted before the putrid proteolytic bacterial fermentation of casein occurs.

The fungus can be found in the mouth and on the skin of healthy people. The conditions under which they become pathogenic for man and animals are not known. This may suggest the presence of carriers, both in humans and animals.

Cox and Tolhurst (1946) showed that torulae in human cerebrospinal fluid, dried at room temperature, remained viable and virulent for at least ten months. Benham (1935) reported the isolation of non-pathogenic strains from the intestinal tracts of normal persons, and he proved these by agglutination tests, to be Torula histolytica. Meyer in 1902 isolated a torula from a nasal tumor of a horse. Harrison in 1928 named this organism Torula nasalis. However, Lodder in 1934 reported it to be identical with Torula histolytica.

In human infection, it is rarely possible to be certain of the portal of entry of the organism into the tissues, or the channels by which it spreads. This field of research has been little explored, and in the absence of other evidence, we can say that human beings, as well as other animals, may be reservoirs of infection from which the disease is

disseminated by contact or by air currents. Most probably Torula histolytica gains access to the body chiefly by way of the lungs, and from there the organisms can spread through the vascular and lymphatic systems, the subarachnoid space, and the perivascular spaces with which it communicates, and also through tissues which are contiguous with a focus. The occurrence of embolic metastasis to the brain is possible. The pathways by which the organism gains entry into the lymphatic glands are less obvious. At one time, it was thought that the organism may reach the glands from the lungs by way of the lymphatic vessels. It is possible from analogy with other diseases, however, that the infection may gain entry to the abdominal glands from the alimentary canal.

Laboratory Investigations. Microbiological studies of Torula histolytica have been made by various workers, but most of these deal with only one or two strains which have been incompletely studied. Fitchett and Weidman (1934) collected about twenty strains and studied their cultural reactions.

Torula histolytica is a yeast-like, non-sporulating, non-mycelial, budding fungus and is characterized by the development of a white capsule both in tissue and in culture. The presence of this capsule is made evident when mixed with a suspension of India ink. Such preparations reveal thick-walled, ovoid to spherical budding cells 5 to 15 microns in diameter. The cell body contains irregular masses or granules. The cell-wall may appear in single or in double contour

according to adjustment of focus.

No endospores are produced and no mycelium is developed. Torulae in stained tissue sections show great variation both in shape and size. In many of these sections the cell bodies show no halo immediately around them, but appear to lie in a wide space which undoubtedly represents the capsular substance.

The buds are usually round or oval, thin walled protuberances which grow and finally separate as new torulae. In some strains, the bud may attain the size of its parent cell without becoming detached. Both large and small-sized organisms produce buds. As a rule only one bud is produced at a time. Cox and Tolhurst (1946) noticed the development of short, straight or curved tubes or hyphae and that these may attain two or three times the length of the parent cell and may remain narrow or become bulbous in places. The same authors made the interesting observation that in tissues there is the occasional production of very large torulae 15 to 30 microns in diameter. They believe these are calcified organisms. The calcified organism appears round with a granular center, and in wet preparations has a glazed and often laminated appearance, differing from that of the normal organism. In both normal and calcified organisms, the capsule is of approximately the same size which suggests that the calcification involves both the cell-body and the capsule. The only record of calcified torulae is that of Sanfelice who described them in a tumor from an ox.

In cultures, Torula histolytica produces small organisms of from one to four microns in diameter, commonly round or oval. The capsule is not so conspicuous in cultures as in the tissues. However, the addition of India ink to a saline suspension shows its presence.

Drouhet and Segretain (1949) made the observation that hyaluronidase caused shrinking and disappearance of the capsule of Torula histolytica in vitro; a swelling of the membrane to twice the shape of the cell and an agglutination of its contents was observed. The optimum conditions for hyaluronidase activity were 33°C. and at a pH of 4.50 - 4.62 in an acetate buffer.

Todd and Hermann (1936) have suggested for Torula histolytica a life cycle in which the various types of organisms seen in slide cells represent different stages of growth. These workers believe they had discovered a sexual stage, and that fusion results in the formation of a single ascospore. If this is the case, it would be of great botanical interest, and would mean that the organism is not a true Torula because it sporulates. Henrici (1941) also reported fusion between the cells.

Torula histolytica grows readily on ordinary media under aerobic conditions. Primary cultures from the cerebrospinal fluid are usually made on enriched media such as blood agar or serum broth. Swift and Bull (1917) used Loeffler's serum for primary cultures, and they stated that no growth could be obtained on agar or in broth until re-

peated subcultures had been made on serum.

On Sabouraud's glucose agar, at room temperature, the colony appears glistening, mucoid, and tan to brown in color. Some authors have commented on the different types of growth on Sabouraud's agar slopes shown by different strains of Torula histolytica; one type is firm and grows only over the area of inoculation, while the other is soft and flows to the bottom of the tube. Most of the strains liquefy gelatin to a slight degree within six weeks and sometimes within three weeks, but occasionally not for nine or twelve weeks. This has been confirmed by Cox and Tolhurst (1946) and was originally described by Benham and Weis (1934).

In liquid media such as broth or peptone water containing sugars, only slight opacity of the liquid is seen. A sediment forms at the bottom of the tube. The fermentative power of Torula histolytica is weak and variable. Some workers (Frothingham, Weis, Rappaport and Kaplan, McGehee and Michelson) have reported no fermentation or no acid or gas in glucose or other sugars.

Crone, de Grout and Wahlin (1937) tested the resistance to heat of their strain of Torula histolytica using 48 hour-old dextrose agar cultures suspended in peptone broth at pH 7.2. They found that the organisms were killed by heating in a water-bath at 50°C. for forty-two minutes. At 60°C. the cells were killed in less than five minutes.

Kuhn (1939) made the observation that the temperature of rabbits injected with torulae rose from the normal of

about 103°F. to between 105°and 107°F.; but mice so injected remained afebrile (normal temperature about 99°F.). He believed that the difference in body temperature might explain the difference in resistance to infection, mice being much more susceptible.

Cultures of Torula histolytica remain viable for many months.

The antigenic structures of Torula histolytica have not yet been established but are being investigated by Cox and Folhurst (1946).

Pathology. It has been emphasized that torulosis is a chronic or at most a subacute infection. It therefore bears some resemblance to tuberculosis in this respect. Also it may infect lungs and meninges, and cause, or be associated with, enlargement of lymphatic glands. However, torulosis is a less virulent and more chronic condition than tuberculosis, and is less productive of severe systemic disturbances.

When a microscopic examination is made of torula infected tissue, evidence may be found of a chronic or at most a subacute lesion, with collections of lymphocytes and plasma cells, epithelioid, giant cells, and connective tissue, with the formation of granulomata. The distinguishing feature of torulosis is the absence of a tissue reaction. Indeed within the brain itself there is usually astonishingly little reaction about the foci, or even about the large torular collections in the lungs. There is little evidence of a histological nature for the production of any toxin by the organism. The

lesions differ also from those produced in tuberculosis, by absence of caseation.

The description of torular meningo-encephalitis by Stoddard and Culter (1916) is classical. Within the subarachnoid space are found inflammatory cells such as lymphocytes and plasma cells. Epthelioid and giant cells may be exceedingly numerous, although sometimes few, and they often contain torulae and at times seem to be disintegrating. Eosinophile cells are rarely found within the brain or subarachnoid space, although they have been described in some other tissues. The meninges show thickening, with young connective tissue and fibroblasts. The subarachnoid space contains fibroblasts. From these, with lymphocytes, plasma cells and torulae in varying proportions, the granulomata are formed. Caseation, so frequently observed in tuberculosis, must be distinctly unusual in torulosis of the meninges, since its presence has been mentioned only by Stoddard and Cutler (1916).

Diagnosis. For the diagnosis of torulosis, sputum, pus, gelatinous exudates or sediment of centrifuged spinal fluid should be examined unstained by placing a small amount under a cover glass. Also, these materials should be mixed with a small amount of India ink and examined under a cover glass before the preparation dries. Specimens should be cultured on Sabouraud's glucose agar at room temperature and blood agar at 37°C. Some infected materials, especially the cerebrospinal fluid, may also be injected intraperitoneally

or intracerebrally into mice to reproduce the disease in these animals. A saline suspension of the culture should be injected into mice to distinguish the pathogenic Cryptococcus neoformans from some of the non-virulent but morphologically similar cryptococci (Benham 1935).

A method for identifying Torula histolytica has been described recently by Mager and Aschner (1946) based on the production of extracellular starch by the organism in a special medium. Yeasts isolated from cases suspected of torulosis are transferred to a medium containing:

Ammonium sulfate	0.1 percent
Magnesium sulfate	0.05 percent
Potassium acid phosphate	0.1 percent
Glucose	1.0 percent
Agar	2.5 percent
Thiamin	0.2 gamma per cc.

Ammonium sulfate serves in this medium both as a source of nitrogen and as a regulator of the acidity necessary for the production of starch. Vitamins other than thiamin are not essential for cultivation of starch-producing yeasts in synthetic media. After some days of incubation at 37°C. the plate is flooded with potassium iodide solution. In the positive cases the streak of growth turns deep blue.

SECTION III

EXPERIMENTAL IN VITRO STUDIES

Schmidt et al. (1949) studied various strains of Cryptococcus neoformans. They showed that the organism grows vigorously in a solution containing glucose, inorganic salts, ammonium salts, and vitamins. Purines and pyrimidines are not essential and amino acids exert merely a slight stimula-

tory effect. In the course of their studies they determined growth by acid titration and by turbidity measurements. Further, they showed that the organism utilized oxythiamin to some extent but not neopyrithiamin. These analogs did not effectively inhibit thiamin utilization by the fungus. The same technique was used in the course of this investigation.

The Agar Diffusion Technique. Preparation of the Agar Plate: 6.5 grams of Sabouraud's dextrose agar were dissolved by heating and stirring in 100 cc. of water. The solution thus formed was autoclaved for fifteen minutes at 15 pounds. About 20 cc. of hot agar were poured into sterile Petri dishes and left standing about an hour to solidify.

Preparation of suspension of Torula histolytica for inoculation of culture media on agar: A broth tube (liver extract) was inoculated with the material obtained from a Sabouraud's agar slant culture of the organism incubated for 24 hours at 37°C. and then allowed to stand for 24 hours at room temperature. The tube was centrifuged and the supernatant fluid was decanted. 10 cc. of sterile saline solution were added to the residue and by shaking well, a homogenous suspension was obtained. The liquid culture medium was inoculated by adding one drop of this suspension to each tube. For the agar diffusion technique, 2 cc. of sterile saline were added instead of 10 cc.

The compounds to be studied were dissolved in water, alcohol or in propylene glycol. 0.5 cc. of the solution of the compound under investigation, in various dilutions as

given in Table I, were mixed with 20 cc. of warm sterile agar. The agar was then poured into the Petri dish. The plates were inoculated with four radial streaks with a sterile loop containing the active culture of Torula histolytica. The plates were then incubated at room temperature. This method can be applied to both water-soluble and water-insoluble drugs.

In all the experiments, the organism was cultivated under the same environmental conditions. Propylene glycol, alcohol, acetone, etc. were found to have no influence whatsoever on the growth of the torula.

Results of in vitro experiments by the agar diffusion technique: Cultures of Cryptococcus neoformans were isolated from local patients admitted to the University Hospital in Baltimore, Maryland. Typical strains were selected for our study. As a matter of fact, minor variations exist between different strains.

The results of these experiments on the inhibition of torula are given in Table I. The readings were made by visual examination five and ten days after incubation. Compounds which proved inhibitory in a concentration of 0.025 mg. per cc. of agar were restudied in a more dilute concentration.

The writer is indebted to Mrs. Norma McElvain, Mrs. Jane Beardsley and to Dr. E. G. Schmidt for performing many of the following in vitro experiments.

TABLE I

The Effect of Various Compounds upon the Growth of *Torula histolytica* by the Agar Plate Diffusion Method.*

Name of Compound Used	Concentration of Drug/cc. of Agar							
	2.5mg/cc		0.25mg/cc		0.025mg/cc		0.005mg/cc	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
A. Thiocyanates								
1. Morpholine thiocyanate	O	O	O	O	O	O		
2. Ephedrine thiocyanate	O	O	O	O	O	O		
3. Hexamethyltetramine thiocyanate	C	C	P	P	O	O		
4. Choline thiocyanate	O	O	O	O	O	O		
5. Barium thiocyanate	O	O	O	O	O	O		
6. Quinaldine thiocyanate	P	O	O	O	O	O		
7. Dithiocyanoaniline	C	C	C	C	C	C		
8. Thiocyanoacetanilide	C	C	C	C	O	O		
9. Thiocyanoaniline	C	C	C	C	O	O		
10. Phenylethanolamine thiocyanate	P	O	O	O	O	O		
11. Styrene dithiocyanate	P	O	P	O	O	O		
12. Potassium thiocyanate	O	O	O	O	O	O		
13. Sodium thiocyanate	O	O	O	O	O	O		

TABLE I (continued)

Name of Compound Used	Concentration of Drug/cc. of Agar							
	2.5mg/cc		0.25mg/cc		0.025mg/cc		0.005mg/cc	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
14.m-Thiocyanobenzoic acid	O	O	O	O	O	O		
15.Butadiene dithiocyanate	C	C	C	C	C	C	O	O
16.Dithiocyanoacetanilide	C	C	C	C	C	C	C	P
17.Dithiocyano alpha-naphthylamine	P	P	P	P	P	P	O	O
B. Antibiotics and allied compounds								
1.Methopterin	O	O	O	O	O	O		
2.Chloromycetin	O	O	O	O	O	O		
3.Hetrazan	O	O	O	O	O	O		
4.Aureomycin Hydrochloride	O	O	O	O	O	O		
5.Zinc Undecylenate	C	C	C	C	O	O		
6.Usnic Acid			O	O	C	O		
7.Bacitracin			O	O	O	O		
8.Darvisul	O	O	O	O	O	O		
9.Hykinone	P	P	O	O	O	O		
10.Synkavite	P	P	O	O	O	O		
11.Vitamin K_5	C	C	C	C	C	C	C	P
12.Dimethyl dichlorosuccinate	C	C	C	C	C	C		
13.Pseudomethyl acetylacrylate	C	C	C	C	C	C	P	P

TABLE I (continued)

Name of Compound Used	Concentration of Drug/cc. of Agar							
	2.5mg/cc		0.25mg/cc		0.025mg/cc		0.005mg/cc	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
14.Subtilin	O	O	O	O	O	O		
15.Undecylenic Acid	C	C	C	C	C	P	O	O
C. Phenolic Compounds								
1.p-Chlorobenzoic Acid	C	C	C	C	P	O		
2.Thymol	C	C	C	C	P	O		
3.Octyl resorcinol	C	C	C	C	C	P		
4.Chlorthymol	C	C	C	C	P	P		
5.Carvacrol	C	C	P	P	O	O		
6.Eugenol	P	P	P	P	O	O		
7.p-Cresol	C	C	P	P	O	O		
8.Hexachlorophene	C	C	C	C	C	C	C	C
D. Alcohols and Ketones								
1.Pentanediol	O	O	O	O	O	O		
2.Propylene glycol	O	O	O	O	O	O		
3.Diacetyl	C	C	C	P	P	O		
E. Aromatic Nitrogen Compounds								
1.Alkyl methyl pyridinium chloride	C	C	C	C	P	P	O	O
2.2-amino-3-methyl pyridine	P	P	O	O	O	O		

TABLE I (continued)

Name of Compound Used	Concentration of Drug/cc. of Agar							
	2.5mg/cc		0.25mg/cc		0.025mg/cc		0.005mg/cc	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
3.Sulfapyridine	C	C	C	C	C	P	O	O
4.Aminopyridine	P	P	O	O	O	O		
5.Eschridine	C	C	C	C	C	C	P	O
6.Pyridine	O	O	O	O	O	O		
7.Germitol	C	C	C	C	P	P	O	O
F. Furane Compounds								
1.NF 1 (a)	C	C	C	P	O	O		
2.NF 45 (b)	C	C	C	C	P	O		
3.NF 67 (c)	O	O	O	O	O	O		
4.NF 84 (d)	P	P	P	P	O	O		
5.NF 5 (e)	C	C	C	C	C	C	P	O
6.Furacin	O	O	O	O	O	O		
G. Dyes								
1.Neutral red	C	C	O	O	O	O		
2.Alizarine red	O	O	O	O	O	O		
3.Methyl red	C	O	O	O	O	O		
4.Methyl orange	O	O	O	O	O	O		
5.Bromthymol blue	C	C	P	P	O	O		
6.Methylene blue	C	C	P	O	O	O	O	O

* O= No inhibition; P= Partial inhibition; C= Complete inhibition

(a) NF 1 = 5-Nitro-2-furaldehyde diacetate.

(b) NF 45= Methyl 5-nitro-2-furfuryl ether.

(c) NF 67= 5-Nitro-2-furaldehyde-2(2-hydroxyethyl) semicarbazone.

(d) NF 84= 5-Nitro-2-furaldehyde semioxamazone.

(e) NF 5 = Methyl 5-nitro-2-furoate.

Liquid Media Method. Each liter of the basal medium contained the following constituents:

Glucose	20	grams
Ammonium sulfate	0.17	"
Ammonium phosphate	1.0	"
Potassium acid phosphate	1.5	"
Sodium chloride	1.0	"
Magnesium sulfate	0.3	"
Calcium chloride	0.2	"
Manganese sulfate	1.0	"
Ferric chloride	1.0	"
Copper sulfate	0.2	"
Sodium borate	0.2	"
Zinc sulfate	0.14	"
Potassium iodide	0.1	"
Molybdic acid powder (85%)	0.02	"
p-Amino benzoic acid	600	gamma
Biotin	3	"
Calcium pantothenate	200	"
Nicotinic acid	200	"
Pyridoxine hydrochloride	600	"
Riboflavin	100	"
Thiamin hydrochloride	200	"
Folic acid	2	"
Choline chloride	1000	"

Ten cc. of beef broth solution (Armour) were inoculated with a loopful of the pure culture taken from a well-grown Sabouraud's agar slant and incubated at 37°C. for 24 hours. The tube was then centrifuged, the fluid decanted and 10 cc. of sterile normal sodium chloride solution added to the residue. The suspension thus formed was counted in a hemocytometer. The counts varied from 4500 to 5500 organisms per cubic millimeter. Each culture tube was then incubated

with a drop of this suspension.

The growth response was measured by acid production and by turbidity. To 5 cc. of the basal medium was added a trace of dry starch which produced a turbidity resembling that in the culture tubes. Five to seven drops of bromthymol blue indicator were added and the pH adjusted to 6.9. This tube was used as a standard for comparison. Each assay tube was then titrated to pH 6.9 with 0.02N sodium hydroxide after the addition of the indicator (McMahan and Snell 1944). Care was taken to prevent self-infection.

This procedure appeared to be accurate since measured amounts of standard lactic acid were quantitatively titrated in this manner.

The titrated, cotton-plugged tubes were then sterilized at 18 pounds of steam pressure for 15 minutes. The heavy, white, adherent cell growth was thereby reduced to a fine suspension. 2 drops of N/1 sulfuric acid were added to each tube and the volumes adjusted to 15 cc. with water. A tube of basal medium treated as above except for the omission of starch was set at 0 in a Klett-Summerson photoelectric colorimeter which contained a No. 66 red light filter (640-700 milli-microns). The contents of each tube were mixed by inversion and read immediately. Serial dilutions of these suspensions, after each reading, gave straight line curves which proved that acidity and turbidity were in direct proportion. The results obtained are recorded in Table II.

Only a few compounds were studied by this method since the added drugs frequently interfered with the titration and turbidity measurements.

TABLE II

The Effect of Various Compounds Studied on the Growth of *Torula histolytica* by the Liquid Medium Method

Compound Used	Days after inoculation	2 mg./cc. of media		0.2 mg./cc. of media		0.02mg./cc. of media	
		cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading
2-amino pyridine	Blank	1.0	3	1.4	6	1.5	7
	5	0.8	6	2.3	21	2.3	42
	9	2.4	14	4.2	47	4.4	65
	14	4.5	26	5.0	83	5.5	83
	19	5.0	36	6.7	100	6.5	86
	22	5.0	39	6.5	95	6.0	95
	26	6.1	51	6.0	109	6.4	119
Germitol	Blank	0.8	13	1.5	27	2.7	21
	5	0.5	0	1.6	2	2.0	0
	9	0	9	-	20	-	0
	14	0	16	-	29	-	23
	19	0	15	-	36	-	26
	22	0	13	-	29	-	22
	26	0	13	-	22	-	18
Sulfa-pyridine	Blank	1.0	7	1.0	7	1.4	7
	4	2.1	30	1.8	30	1.4	25
	7	2.1	56	3.2	62	3.6	56
	12	3.0	87	3.8	81	3.6	84
	16	4.0	78	4.1	73	4.0	74
	21	4.2	88	3.8	65	4.0	68
	26	4.5	133	3.8	83	3.9	108

TABLE II (continued)

Compound Used	Days after inoculation	0.2mg/cc of media		0.04mg/cc of media		0.004mg/cc of media	
		cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading
Eschridine	Blank	1.7	4	1.1	5	1.4	7
	4	2.4	35	1.8	27	2.3	34
	7	2.9	75	1.9	51	3.5	83
	12	3.6	61	3.0	86	3.5	78
	16	2.1	17	4.7	96	3.9	102
	21	4.1	80	3.6	128	4.0	88
	26	5.0	82	6.0	123	5.5	124
Synkavite	Blank	1.1	7	1.4	7	1.4	7
	4	1.1	3	1.7	1	2.0	23
	7	1.4	11	1.8	11	3.3	43
	12	1.4	9	1.8	17	3.4	58
	16	1.7	13	1.7	8	4.0	61
	21	2.1	12	2.1	20	3.7	88
	26	1.8	9	1.5	8	4.5	83
Hykinone	Blank	1.3	6	1.2	7	1.3	7
	4	1.2	0	1.4	6	1.9	22
	7	1.3	12	2.0	19	3.5	49
	12	1.5	10	3.0	33	3.7	52
	16	1.6	9	2.8	34	4.0	71
	21	1.2	11	4.9	152	3.9	73
	26	2.0	9	4.6	187	4.5	98
		1 mg/cc of media		0.1 mg/cc of media		0.01 mg/cc of media	
		cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading
Biacetyl	Blank	1.9	7	1.6	8	1.1	7
	4	2.0	8	1.9	20	2.0	30
	7	2.9	52	3.2	75	4.3	84
	12	3.1	35	3.8	78	4.2	79
	16	3.4	30	4.2	67	4.1	87
	21	2.3	13	4.5	81	4.3	80
	26	5.0	60	5.5	112	6.0	150

TABLE II (continued)

Compound Used	Days after inoculation	0.2 mg/cc of media		0.04 mg/cc of media		0.004 mg/cc of media	
		cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading	cc 0.02N NaOH	color reading
Vitamin K_5	4	0.5	28	0.4	7	0.4	5
	9	0.5	24	0.5	4	0.8	23
	19	0.5	22	0.7	4	4.3	85
	23	0.9	29	0.5	7	3.5	60
	27	0.9	21	0.8	6	4.3	79
	31	0.7	27	0.6	7	4.1	69
	34	0.8	23	0.5	4	4.4	83

CONTROL TUBES

No Drug Added but Inoculated

Tube No.	Days after Inoculation	cc. 0.02N NaOH	Colorimeter reading
1	4	1.6	34
2	7	3.4	56
3	12	4.8	55
4	16	5.4	87
5	21	5.9	116
6	26	5.8	135

SECTION IV

PHARMACOTHERAPEUTICS

A successful treatment for human torulosis is as yet unknown. In the course of this investigation an attempt has been made to find an effective drug to combat the disease. The course of torulosis is usually one of steady progression. However, some patients do remit and improve and become symptom free; but sooner or later, the symptoms recur. As a matter of fact, the remissions may be prolonged to the ex-

tent of six months.

The most difficult problem in the treatment of torulosis is that of the meningitis and encephalitis. Various drugs and compounds have been employed orally, subcutaneously, intramuscularly, intravenously, or by injection into the spinal subarachnoid space after withdrawal of cerebrospinal fluid.

So far no definite success has been obtained. The writer believes that the failure of all these drugs is due to inaccessability of the drug to the organisms because of its thick capsule. This is similar to the difficulty encountered in the treatment of tuberculosis because of the waxy capsule around the cell. Also the penetration of drugs into the central nervous system is difficult.

Vaccine therapy has been employed without success. Iodides, known to exert a favourable influence on some other fungus diseases, have been used extensively in torulosis. As much as 26 grams of potassium iodide were given daily by mouth, as well as by intrathecal injections on alternate days (Shapiro and Neal, 1925). Favourable results were not recorded except in some of the localized infections.

The sulfonamide group of drugs has been used recently but usually with disappointing results. Sulfapyridine proved to be effective <u>in vitro</u> but ineffective <u>in vivo</u>. Marshall and Teed (1942) claimed great benefit from sulfadiazine. The dose employed was half a gram every four hours, continued for six weeks for a child aged nine years.

Quinine and organic arsenical compounds have been tried without apparent benefit.

Torula histolytica seems to be highly resistant to penicillin. Stone and Sturdivant (1929) found that gold sodium thiosulfate inhibited the growth of cultures and therefore gave it to their patients intravenously. Gentian violet was also found to have an inhibitory effect and was used intravenously and intraspinally. Hexamethylamine was also used. The results were uniformly disappointing.

Shapiro and Neal (1925) made the observation that acriflavine in a dilution 1:1000 had a marked inhibitory effect on cultures isolated from their patients. The same authors found sodium iodide, quinine sulfate, sodium salicylate, colloidal silver, magnesium sulfate, and tricresol to have an inhibitory action in the same concentration as acriflavine. However, in vivo therapy proved ineffective. Sodium thiosulfate and colloidal copper were used by Cox and Tolhurst (1946) for their patients with no success. Although copper is of value in certain fungus diseases of plants, it has no appreciable effect on the patient's condition. Stone and Sturdivant (1929) showed that Torula histolytica can grow well on media containing colloidal copper. Shapiro and Neal (1925) injected colloidal silver and immune rabbit's serum intraspinally without success. Lynch and Rose injected 20 cc. of 1:1000 mercurochrome but their patient died sixteen hours later.

Stone and Sturdivant (1929) showed that X-rays have an

inhibitory action on cultures of Torula histolytica. Although there was no evidence that torular meningitis was benefited by deep X-ray therapy, it relieved the enlargement of lymph glands associated with torulosis. Pyretotherapy has been suggested owing to the fact that cultures kept at 105° and 107°F. for seven and six days respectively do not survive.

Crystalline actidione, an antibiotic from Streptomyces griseus, was shown by Leach et al. (1947) to inhibit Cryptococcus neoformans in concentrations as low as 0.0002 milligrams per cc. This high order of activity suggests its possible usefulness in the treatment of the disease. Actidione, however, is very toxic when used in vivo.

Geiger (1948) in a series of experiments with unsaturated ketones reported inhibition of cultures of Cryptococcus neoformans and he suggested that the antifungicidal action of these compounds was a result of their ability to react with sulfhydryl groups of the enzymes in the organism. Landy et al. (1948) described the antifungal activity of partially purified bacillomycin as measured by the agar-plate method. This antibiotic is isolated from Bacillus subtilis. It possesses striking antifungal activity and almost complete lack of antibacterial action.

The blockage of the thyroid hormone synthesis by thioureas, presumably by interference with enzyme activity, raises the possibility that this drug may have other properties based upon this action. Danowski and Tager (1948) studied

the influence of thiourea on the growth of Cryptococcus hominis. They came to the conclusion that thiourea suppressed in a varying degree the in vitro growth of several pathogenic fungi and that the presence of blood or serum minimized this action. The same authors further showed that thiourea failed to influence the course of torulosis in mice.

Infection of Animals. In these experiments mice were injected with strains of Torula histolytica obtained from patients admitted to the University of Maryland Hospital. Ten cc. of beef broth were inoculated with a loopful of the pure culture taken from a well-grown Sabouraud's agar slant. The tube was incubated at 37°C. for 24 hours, and then at room temperature for 24 hours. The tube was then centrifuged, the fluid was decanted and 10 cc. of sterile normal saline were added to the residue. A homogenous mixture was obtained by shaking the tube. Mice were anesthetized with ether by putting the animals in a jar with cotton impregnated with ether. Following the intracerebral injection of torula by the usual method, death occurred in from seven to twenty days. Experiments showed marked variation in individual resistance. Mice which died in less than fifteen days generally showed, post mortem, no obvious evidence of pathological lesions. Examination of mice which died after fifteen days revealed torular infection localized in the central nervous system. Histological specimens were made from different organs and the following report was submitted by Dr. D. L. Reiman of the Department of Pathology, Medical

School, University of Maryland.

B 929 - Liver: An easily recognized lobular architecture was seen in this section. The portal areas were infiltrated by lymphocytes. There was no cirrhosis or hemorrhage. The hepatic cells showed the variability in conformation and chromaticity usually associated with a regenerative process. Evidence of congestion was noted. Areas of autolysis were observed in this specimen.

Kidney: The glomeruli were free of hemorrhage, scarring and hyperplasia. The cells of the tubules were not remarkably changed. There was no evidence of pelvic infection. The arterioles were well preserved.

Lung: There was no consolidation in this organ. The alveolar and bronchiolar constituents were normal in appearance.

Pancreas: Only the acinar elements of this organ were demonstrated. There was no evidence of neoplasia, hemorrhage, necrosis or fibrosis.

Mucous gland: A mucous gland was included in this group. There were no abnormalities in this organ.

B 930 - Liver: This organ displayed a well preserved lobular architecture. There was no evidence of cirrhosis, degeneration or necrosis.

Lung: The alveolar and bronchiolar structures were normal in this organ except for congestive changes. There was no consolidation.

Brain: A single group of torula was noted beneath the

pia mater covering the cerebellum. There was no histological evidence of granulomatous response to the organism. The deuronal and glial elements were not effected.

B 931 - Liver: A well preserved lobular architecture was noted in this organ. The portal areas were free of cirrhosis and hemorrhage. The hepatic cells were granular, well delineated, and possessed of normal appearing nuclei. The central areas were not remarkable.

Kidney: The capsular part of the glomeruli was composed of cuboidal, eosinophilic cells that simulate tubercle cells. The capsular space was empty. There was no evidence of inflammation. The tubular structure appeared normal. The arterioles were free of sclerosis.

Lung: There was no consolidation or granulomatous inflammation in this organ. Evidence of consolidation was noted. The bronchioles were patent and well preserved.

Brain: Slight fibrosis was noted in focal areas of the pia mater. Small areas of necrosis were scattered through the cerebral cortex and cerebellum. There was no inflammatory response in the tissues which surround the lesions. Nurulae were seen.

B 932 - Liver: This organ was free from significant changes. In all respects, it compared with the above specimens.

Brain: Minimal inflammatory reaction was noted in certain portions of the pia mater. Areas of necrosis which contained parasites were seen in the cerebral cortex and

cerebellum.

Lung: Evidence of congestion was observed in this organ. The bronchioles were normal in appearance. There was no evidence of consolidation.

B 933 - Liver: This organ was free of degeneration, necrosis, and hemorrhage. There was no congestion. A normal lobular architecture was observed.

Kidney: The parietal layer of Bowman's capsule was cuboidal. The capsules were empty. There was no evidence of inflammation. The cells of the tubules were granular and possessed of normal appearing nuclei. There was no evidence of pelvic infection. The arterioles were free of sclerosis.

Brain: In the cerebellum, a dense aggregate of lymphocytes and polymorphonuclears was seen. In this area innumerable torulae were seen. There was no granulomatous response. The pia mater was slightly thickened.

B 934 - Kidney: There was no evidence of endothelial or capsular hyperplasia. The capillaries were patent. There were no tuft adhesions or scarring. The capillaries were empty. The parietal layer of the glomerular capsule was cuboidal. The cells of the tubules were pale, granular and somewhat fragmented. This was the result of post mortem change. The arterioles were free of sclerosis. There was no evidence of pelvic infection. A hilar artery was seen which contained foreign cells. The significance of these was questioned.

Liver: This organ was autolyzed. A normal architecture was recognized. There was no evidence of necrosis or cirrhosis.

Pathogenicity for mice had been confirmed in our experiments and all the mice infected had shown the signs of the disease. In about five days the animals became restless and in about ten days, the skull showed a swelling over the top of the head and later the head became spindle shaped. This sign was a sure test that the mouse had caught the infection.

Torulosis in experimental animals closely resembles the human disease though in man the course of torulosis is more prolonged.

Our strains of torula were all pathogenic to mice. However, variation in virulence existed between different strains.

Closer examination of the pathological report of animals inoculated intracerebrally showed that the organism did not spread into the other organs such as the liver and kidney because there was no time for dissemination owing to the quick fatal end of meningo-encephalitis.

Toxidity Studies. In the in vitro experiments, certain compounds were found which proved very effective in inhibiting the growth of Torula histolytica. These compounds undoubtedly were worth a trial in vivo. However, the toxicity and the effective doses of these compounds had to be determined first. Among the compounds which inhibit the growth of cultures in vitro, the following were tested for their therapeutic effect on infected animals:

1. Eschridine (4-4'-ethylcyclohexylmethyl pyridine)
2. Vitamin K_5 (4-amino-2-methyl-1-naphthol hydrochloride)
3. Biacetyl
4. Methyl-5-nitro-2-furoate (NF 5)
5. Pseudo methyl acetylacrylate
6. Hexachlorophene (2,2'-methylene bis (3,4,6-trichlorophenol)
7. Dithiocyanoacetanilide
8. Marpharsen (2-amino-4-arsenophenol)

The toxicity of these sompounds, with the exception of mapharsen and Vitamin K_5, was determined by intraperitoneal injection into mice.

Compounds 1, 3, 4, 5, 6, and 7 on intraperitoneal injection gave rise to very definite toxicological symptoms. Eschridine (I) in toxic doses (20 mg. per 100 grams body weight) caused within half an hour after administration definite hypersensitivity, premonitory symptoms of hyperirritability, hyperreflexia, tremors changing to convulsions of very short duration, followed by weakness and death. Death which followed within fifteen minutes after injection of a lethal dose appeared to be due to respiratory failure. The heart continued to beat after respiration had ceased.

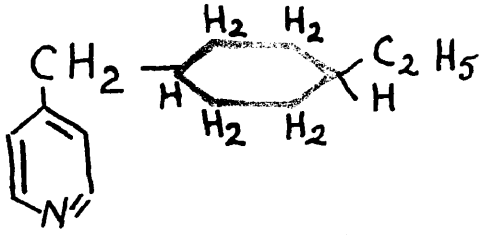
Pseudo methyl acetylacrylate (VII) in a dose of 7.5 mg. per 100 grams body weight produced convulsions, followed by a short depression, ending in death within a few minutes. The respiration was slow.

After the intraperitoneal administration of dithiocyanoacetanilide to mice in a dose of 6.2 mg. per 100 grams body weight, tremors and skeletal muscle fibrillations

were noted within a short time. With higher doses convulsions and respiratory paralysis followed quickly. The heart continued to beat slowly but forcibly after the cessation of the respiration.

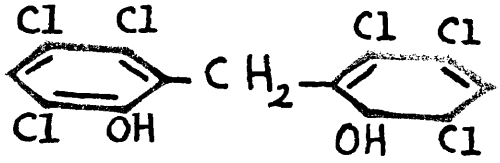
Methyl-5-nitro-2-furoate (IV) in a dose of 37.5 mg. per 100 grams body weight of mice showed symptoms similar to those produced by dithiocyanoacetanilide.

I.



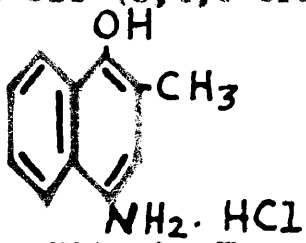
Eschridine
4-(4'-Ethylcyclohexylmethyl) pyridine

II.



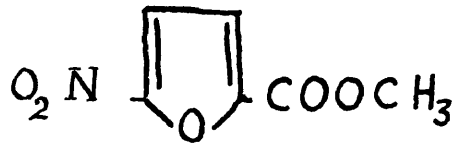
Hexachlorophene
2,2'Methylene bis (3,4,6-trichlorophenol)

III.



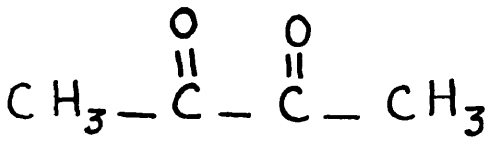
Vitamin K_5
4-Amino-2-methyl-1-naphthol hydrochloride

IV.



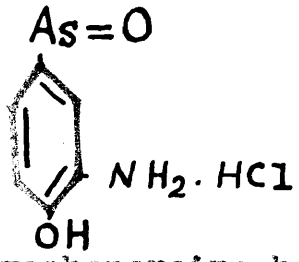
Methyl-5-nitro-2-furoate (NF 5)

V.



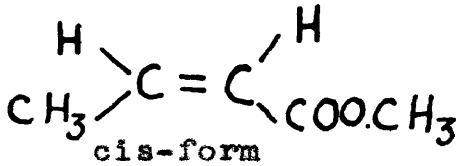
Biacetyl
2,3-Butanedione

VI.



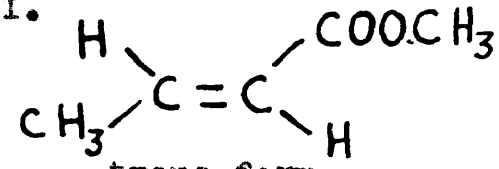
Mapharsen - (Oxophenarsine hydrochloride)
2-Amino-4-arsenophenol

VII.



cis-form

VIII.



trans-form

Pseudo Methyl Acetylacrylate

Methyl acetylacrylate can exist in the cis (VII) and trans-forms (VIII). Pseudo methyl acetylacrylate is the cis-form (VII).

The LD_{50} of each compound was determined by intraperitoneal administration into mice. Those compounds which were insoluble in normal saline were put in solution either in propylene glycol or in olive oil. The results are recorded in Table III.

An estimation of the LD_{50} for each compound was obtained by plotting the data from Table III on a semi-logarithmic paper, the ordinates being percent mortality and the abscissae the dose in milligrams per 100 grams of animals.

The approximate LD_{50} is the dose corresponding to the

point where the curve crosses the 50% mortality line. These approximate LD_{50}'s are given in Table IV.

TABLE III

Toxicity Data by Intraperitoneal Administration into Mice

Compound Studied	Dose mg./100 grams	Number of animals injected	Percent Mortality
Biacetyl	175	8	100%
	150	8	75
	125	8	37
	100	8	0
	87.5	8	0
	75	8	0
Methyl-5-nitro-2-furoate (NF 5)	37.5	9	100%
	30.0	9	77
	22.5	9	44
	15.0	9	22
	7.5	8	0
Pseudo methyl acetylacrylate	8.75	8	100%
	7.5	6	100
	6.25	6	83
	5.0	6	66
	3.75	6	33
	2.5	6	0
Eschridine	20.0	8	100%
	10.0	8	87
	7.5	8	62
	5.0	8	25
	2.5	8	0
	1.25	8	0
Dithiocyano-acetanilide	6.25	8	100%
	5.0	8	75
	3.75	8	37
	2.5	8	12
	1.25	8	0
Hexachlorophene	4.5	8	100%
	3.75	8	87
	3.0	8	75
	2.25	8	62
	1.5	8	37
	0.75	8	0

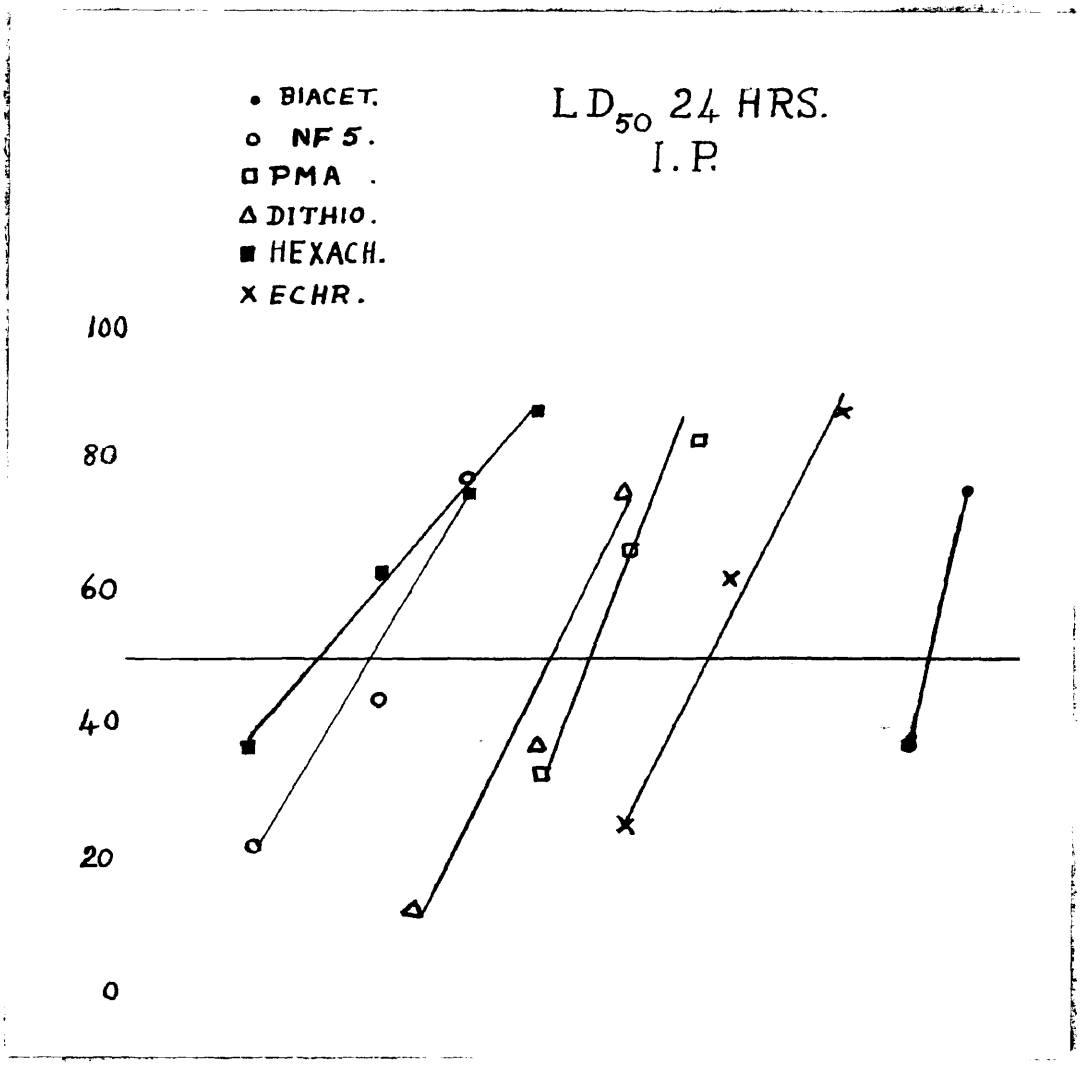

• BIACET.
o NF 5.
□ PMA .
Δ DITHIO.
■ HEXACH.
X ECHR.
LD50 24 HRS.
I.P.
100
80
60
40
20
0

TABLE IV

The Approximate LD_{50} (24 hours) of the Compounds Studied by Intraperitoneal Injection

Compound Studied	LD_{50} in mg. per kilogram body weight
1. Biacetyl	1320.0
2. Methyl-5-nitro-2-furoate	220.0
3. Pseudo methyl acetylacrylate	44.0
4. Eschridine	65.0
5. Dithiocyanoactanilide	40.0
6. Hexachlorophene	18.0

Trial of Treatment. Mice are very susceptible to torulosis and most of them take the infection. We made this study on LCSa strain of mice obtained from Dr. Figge's colony.

After the animals were inoculated intracerebrally with the torula organism, they were divided into groups of ten. Treatment was instituted on the following day. However, one group was used as a control. The maximum dose was determined from the LD_{50} and was injected daily intraperitoneally.

Decreasing doses of the drug investigated were given to the remaining groups of infected mice.

The compounds which were not soluble in saline solution were dissolved in propylene glycol or in warm olive oil.

The syringe and needle were sterilized in boiling water before each group of mice was injected. The results obtained are recorded in Tables V,VI,VII,VIII,IX,X,XI and XII.

It is obvious from the data given in these tables that there was no difference in the survival time between the control mice and those which had been injected with the drugs. Therefore, these drugs did not influence the course of the disease.

TABLE V

Effect of Eschridine on the Survival of Mice Inoculated with Torula histolytica

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	2	3	4	1
Group I 0.5% - 0.1 cc.	5	3	2	-
Group II 0.3% - 0.1 cc.	4	2	3	1
Group III 0.1% - 0.1 cc.	2	3	3	2
Group IV 0.05% - 0.1 cc.	3	2	4	1

TABLE VI

Effect of Vitamin K_5 on the Survival of Mice Inoculated with Torula histolytica

10 mice in each group

Daily Dose	Deaths in Weeks			
	First Week	Second week	Third week	Fourth week
Control	2	4	4	-
Group I 1% - 0.2 cc.	5	3	2	-
Group II 0.5% - 0.2 cc.	4	4	2	-
Group III 0.3% - 0.2 cc.	3	2	3	2
Group IV 0.1% - 0.2 cc.	3	2	4	1

TABLE VII

Effect of Biacetyl on the Survival of Mice Inoculated with Torula histolytica

8 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	2	3	3	-
Group I 3% - 0.3 cc.	5	2	1	-
Group II 2% - 0.3 cc.	4	2	2	-
Group III 1% - 0.3 cc.	3	2	3	-
Group IV 0.5% - 0.3 cc.	2	3	2	1

TABLE VIII

Effect of Methyl-5-nitro-2-furoate on the Survival of Mice Inoculated with Torula histolytica

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	3	2	3	2
Group I 1% - 0.1 cc.	4	2	4	-
Group II 0.5% - 0.1 cc.	2	5	3	-
Group III 0.2% - 0.1 cc.	2	3	3	2
Group IV 0.1% - 0.1 cc.	2	2	4	2

TABLE IX

Effect of Pseudo methyl acetylacrylate on the Survival of Mice Inoculated with Torula histolytica

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	2	3	4	1
Group I 0.5% - 0.1 cc.	4	2	3	1
Group II 0.3% - 0.1 cc.	3	2	5	-
Group III 0.2% - 0.1 cc.	3	2	3	2
Group IV 0.1% - 0.1 cc.	2	3	3	2

TABLE X

Effect of Mapharsen on the Survival of Mice Inoculated with *Torula histolytica*

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	3	4	2	1
Group I 0.1% - 0.1 cc.	4	3	3	-
Group II 0.05% - 0.1 cc.	5	3	2	-
Group III 0.02% - 0.1 cc.	2	4	2	2

Solutions of mapharsen were changed frequently to avoid changes in the active principal.

TABLE XI

Effect of Dithiocyanoacetanilide on the Survival of Mice Inoculated with *Torula histolytica*

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	3	2	4	1
Group I 0.5% - 0.1 cc.	4	3	2	1
Group II 0.3% - 0.1 cc.	3	3	3	1
Group III 0.2% - 0.1 cc.	2	4	3	1
Group IV 0.1% - 0.1 cc.	2	3	3	2

TABLE XII

Effect of Hexachlorophene (G-11) on the Survival of Mice Inoculated with Torula histolytica

10 mice in each group

Daily Dose	Deaths in Weeks			
	First week	Second week	Third week	Fourth week
Control	2	2	4	2
Group I 0.2% - 0.1 cc.	4	3	3	-
Group II 0.1% - 0.1 cc.	3	3	3	1
Group III 0.05% - 0.1 cc.	2	3	4	1
Group IV 0.02% - 0.1 cc.	2	3	3	2

SECTION V

DISCUSSION

From our studies on Torula histolytica, a variety of compounds were found to possess a remarkable inhibitory action on the growth of this fungus in vitro. They represent a diverse group of substances among which vitamin K_5 (4-amino-2-methyl-1-naphthol hydrochloride), biacetyl, thymol, octyl resorcinol, hexachlorophene, p-chlorobenzoic acid, alkyl methyl pyridinium chloride, eschridine, sulfapyridine, dithiocyanoacetanilide, methyl-5-nitro-2-furoate and pseudo methyl acetylacrylate were found to be most effective.

No single theory is adequate to explain the inhibitory power of such diverse compounds. It has been suggested that

the ability of certain hydroquinones, quinones, and their nitrogen analogs to inhibit enzymes may result from their ability to react with sulfhydryl groups. Such interference with essential enzymes would inhibit growth of the fungus. Also these compounds are effective hydrogen acceptors and may thus interfere with oxidation-reduction cycles essential to life and reproduction of the organism. Tawab and Krantz (1949) made the observation that certain organic thiocyanates used inhibit the cytochrome oxidase and reductase. This may be the explanation for the inhibitory action of dithiocyanoacetanilide on the torula organism. Compounds such as thymol, octyl resorcinol, p-chlorobenzoic acid, hexachlorophene, etc. are probably protoplasmic poisons. Alkyl methyl pyridinium chloride and other quaternary ammonium compounds exert their fungicidal action by means of a lowering of the surface tension. There is evidence that furacin derivatives diminish the activity of tissue dehydrogenases. In most cases, however, an explanation of the apparent molecular specificity exhibited within a series of analogous compounds is not available at present. Unfortunately, many of the compounds which appeared promising <u>in vitro</u>, are very toxic.

The therapeutic doses employed were based on the LD_{50} of the compounds as determined in this work. Approximately one-third of the LD_{50} per mouse was considered the maximum dose and injected daily into each mouse to prevent death due to the drug. This may not produce blood levels of the compounds sufficiently high to inhibit the growth of the fungus. Higher levels likely would have been too toxic.

The almost complete loss of activity of these compounds *in vivo* suggests that the serum and blood proteins, or other colloids might be involved in this inactivation. Also, it is theoretically possible that the blood merely provides a medium sufficiently enriched to accelerate growth and this masks any inhibition by chemical agents. Perhaps the chemical agent, in the presence of blood, is destroyed or some portion of the molecule is either combined with some other substance or is altered sufficiently to deprive the compound of its activity.

Further the thick capsule and the inaccessibility of the drug to reach the organism may account for this failure to influence the progress of the disease. The marked affinity of *Torula histolytica* to the nervous tissue complicates the matter because the brain tissue is known for its difficult penetration by drugs. The relative efficiency of such agents must be dependent upon their mechanical affinity for lipoid substances on the one hand, and for the remaining body constituents, i.e. principally water, on the other hand. Their efficiency is, therefore, dependent upon their partition coefficient which determines their distribution in a mixture of water and lipoid substances. All chemical substances which are soluble in fats and fat-like bodies must exert an action on living protoplasm, insofar as they can become distributed in it. However, in the case of torula, the thick capsule does not allow any penetration.

SECTION IV

SUMMARY

An extensive series of organic compounds was examined for their *in vitro*, inhibitory action on *Torula histolytica*. Two methods for studying inhibition of the organism were employed, an agar-diffusion technique and a liquid medium method. The following compounds were found to be the most effective inhibitors of *Torula histolytica*:

Biacetyl
Vitamin K_5
Pseudo methyl acetylacrylate
Hexachlorophene
Eschridine
Dithiocyanoacetanilide
Butadiene dithiocyanate
Octyl resorcinol
p-Chlorobenzoic acid

Data as to the toxicity of the first six compounds were determined in terms of LD_{50}'s. Mice were infected intracerebrally with *Torula histolytica*. The foregoing compounds were injected into these mice in order to study their possible therapeutic value. However, these compounds failed to influence the course of torulosis induced in mice.

SECTION VII

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