A comparative study was first made of the effect of isolated and purified antibiotics upon the growth of *E. coli ds* (Table 2). A highly purified preparation of streptomycin and a crude preparation of a streptomycin-like material were compared with two forms of purified streptothricin. The latter antibiotic was used, first, because it is similar in antibacterial and in certain other properties to streptomycin, and second, because certain organisms may produce a mixture of streptomycin and streptothricin (*5*). The results obtained show that, whereas the two forms of streptomycin supported good growth of *E. coli ds*, the streptothricin preparations did not permit any growth of this culture.

TABLE 2

EFFECT OF DIFFERENT ANTIBIOTICS UPON THE GROWTH OF A STREPTOMYCIN-DEPENDENT STRAIN OF *E. coli**

Incubation of culture (hrs)	Antibiotic added				
	No streptomycin	Pure streptomycin (10 μg/ml)	Crude streptomycin-like material (10 μg/ml)	Streptothricin (34 μg/ml)	Streptothricin (63 μg/ml)
240	0	27	30	0	0
384	0	27	30	0	0

* Growth of organism in nutrient broth is expressed as turbidimetric readings.

A comparative study was next made of crude culture filtrates of several antibiotic-producing actinomycetes (Table 3). Only the streptomycin-producing filtrate (3463) permitted the growth of *E. coli ds*; the supplementary addition of streptomycin to these cultures resulted in a more rapid initiation of the growth of the organism. The culture filtrates of the other three organisms, which did not produce streptomycin, did not permit the growth of *E. coli ds*; these filtrates included 3495, a mutant obtained from the streptomycin-producing *S. griseus*, but no longer producing any streptomycin; 3527, a grisein-producing culture; and 3530, a streptothricin-producing culture. The addition of streptomycin to these culture filtrates favored the growth of *E. coli ds*, thus indicating that the antibiotics found in the culture filtrates of these organisms did not interfere with the growth-promoting effect of streptomycin; at most, there was a delay, as in the case of 3527, due, no doubt, to the initial inhibiting effect of the grisein produced by this culture upon the growth of the *E. coli ds*.

Results similar to those reported in this paper have been obtained recently by R. J. Canderlinde and D. Yegian, of the Ray Brook State Tuberculosis Hospital, using an agar-streak method for the growth of streptomycin-dependent strains of different bacteria.

TABLE 3

EFFECT OF CRUDE CULTURE FILTRATES OF DIFFERENT ACTINOMYCETES ON THE GROWTH OF A STREPTOMYCIN-DEPENDENT STRAIN OF *E. coli*

Incubation of culture (hrs)	Pure streptomycin		Filtrate 3463†		Filtrate 3495‡		Filtrate 3527§		Filtrate 3530‖	
	Cells alone	Cells + SM*	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM	Filtrate alone	Filtrate + SM
24	0	0	0	0	0	0	0	0		
48	0	12	0	13	0	10	0	0		
74	0	16	1	19	0	14	0	0		
96	0	19	12	21	0	19	0	0		
120	0	20	17	21	0	21	0	1		
264	0	15	19	20	0	21	0	20	0	29
336	0	20	19	23	0	29	0	21	0	34

* Cells = suspension of streptomycin-dependent bacterial culture 8 hrs old and containing 480 visible cells/ml. Where the culture filtrates were used, the filtrate alone indicates that the antibiotic was present only in the form of the culture filtrate; filtrate + SM indicates that purified streptomycin (10 μg/ml) was also added.

† Streptomycin-producing *S. griseus* culture; final diluted broth contained 6.4 μg/ml of streptomycin.

‡ Mutant obtained from streptomycin-producing *S. griseus* culture and not producing any streptomycin; diluted broth contained 1,000 *S. aureus* units/ml.

§ Grisein-producing culture gave 388 grisein units/ml in diluted broth.

‖ Streptothricin-producing cultures gave a dilution of 12–47 units of streptothricin/ml of broth.

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Precipitin Reactions in Experimental Histoplasmosis and Blastomycosis

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Skin tests and complement-fixation reactions have been used as diagnostic aids in suspected cases of histoplasmosis and blastomycosis. The antigens employed consisted mainly of either the filtrates of broth cultures of the mycelial phase or suspensions of the yeast-like forms of the causative organisms. Recent studies by various workers have shown that the former, which is the saprophytic form grown at room temperature, was nonspecific for infections caused by the homologous fungus. The latter, which is the parasitic form when grown at 37° C, appeared to be more specific as an antigen in the complement-fixation reaction than in the skin tests.

Investigations on the precipitin reaction in histoplasmosis (*3*) and blastomycosis (*1, 2*) have been limited

and the results inconclusive. The work presented here is a report on the demonstration of precipitins in the sera of animals inoculated with either *Histoplasma capsulatum* or *Blastomyces dermatitidis*. The antigens used in this study were filtrates of broth cultures of the mycelial phase of *H. capsulatum* or *B. dermatitidis* (hereafter referred to as histoplasmin and blastomycin, respectively) yielded 2 fractions, the first by simply adjusting the reaction to pH 4.2 or 11.2 and the second by the addition of 1 or 2 volumes of ethanol.

In this manner 4 fractions were obtained. Of the 2 fractions isolated at pH 4.2, one, designated fraction 1, was shown by chemical tests to be protein-like in nature. The second, finally obtained by the addition of 1 volume

TABLE 1

RESULTS OF PRECIPITIN TESTS PERFORMED ON THE SERA OF RABBITS INOCULATED WITH *H. capsulatum* OR *B. dermatitidis**

Time after inoculation of rabbits	Antigen dilutions	Antigens (No. of sera showing pos. reactions/No. of sera tested)											
		Histoplasmin		Blastomycin		Fractions of histoplasmin							
						1		2		3		4	
(weeks)		H†	B‡	H	B	H	B	H	B	H	B	H	B
2	Undil.	6/7	1/7	6/7	1/7								
	1:10	6/7	0/7	6/7	0/7	7/7	0/7	7/7	0/7	6/7§	0/7	4/7	0/7
	1:100	3/7	0/6	2/7	0/6	7/7	0/7	7/7	0/7	6/7	0/7	4/7	0/6
	1:1,000	0/7	0/6	0/7	0/6	5/7	0/7	7/7	0/7			3/7	0/6
	1:2,000							7/7	0/7				
3	Undil.	6/7	5/6	4/7	5/6								
	1:10	7/7	0/4	5/7	0/4	7/7	2/6	7/7	1/6	5/7	0/4	4/7	0/4
	1:100	3/6	0/4	2/7	0/4	7/7	1/4	7/7	0/6	5/7	0/4	3/7	0/4
	1:1,000	0/6	0/4	0/7	0/4	5/7	0/4	6/7	0/6			1/7	0/4
	1:2,000							4/7	0/6				
5	Undil.	5/6	5/6	1/6	6/6								
	1:10	2/6	0/6	1/6	1/6	5/6	3/6	1/6	1/6	2/6	1/6	0/6	0/6
	1:100	0/6	0/6	0/6	0/6	1/6	1/6	1/6	1/6	2/6	0/6	0/6	0/6
	1:1,000					0/6	0/6	1/6	1/6			0/6	0/6
	1:2,000							1/6	0/6				
7	Undil.	4/6	5/6	0/6	5/5								
	1:10	2/6	1/5	0/6	3/5	2/6	1/5	0/6	0/5	0/6	0/5	0/6	0/5
	1:100	0/6	0/5	0/6	0/5	0/6	0/5	0/6	0/5	0/6	0/5	0/6	0/5
	1:1,000					0/6	0/5	0/6	0/5	0/6	0/5		
9	Undil.	2/6	2/4	0/6	4/4								
	1:10	1/6	1/4	0/6	1/4	0/6	0/4	0/6	0/4	0/6	0/4	0/6	0/4
	1:100	0/6	0/4	0/6	0/4	0/6	0/4	0/6	0/4	0/6	0/4	0/6	0/4
	1:1,000					0/6	0/4						

* Sera collected prior to inoculation and 1 week after inoculation were negative throughout; sera obtained from 3 normal rabbits at each bleeding were negative throughout.

† Sera obtained from rabbits inoculated with *H. capsulatum*.

‡ Sera obtained from rabbits inoculated with *B. dermatitidis*.

§ A prozone reaction occurred with one of the sera in this group.

and 4 fractions isolated from the filtrates of broth cultures of *H. capsulatum*. Two groups of rabbits were used. One group was inoculated intravenously with the yeast-like phase of *H. capsulatum* and the other with that of *B. dermatitidis*. Three normal rabbits served as controls throughout the experimental period. All the animals were bled prior to inoculation and at weekly or biweekly intervals thereafter, and their sera examined for precipitating antibodies.

The fractionation of histoplasmin was carried out as follows: Two precipitates were obtained by adjusting the reaction to pH 4.2 and pH 11.2 after adding 1 volume of 95% ethanol to the histoplasmin. The precipitates were removed by centrifugation and subsequently dissolved in distilled water. Aqueous solutions of each of ethanol and designated fraction 2, was polysaccharide-like in nature. Fractions 3 and 4 were isolated at pH 11.2. The chemical nature of fraction 3 has not yet been determined. Fraction 4, which was precipitated after the removal of fraction 3 by the addition of 2 volumes of ethanol, was polysaccharide-like in nature.

Precipitins were demonstrated in the sera of the infected animals by means of the so-called "ring test." One-tenth ml of antigen was carefully layered over 0.1 ml of serum in small serological tubes and the presence or absence of a precipitate noted after the tubes had stood at 37° C for 2 hrs. Only those tests showing a definite precipitate at the interface were regarded as positive. All others were recorded as negative.

In Table 1 the sensitivity and specificity of histo-

plasmin, blastomycin, and fractions 1, 2, 3, and 4 from histoplasmin have been compared. The antigenic content of each solution was not determined by weight. Specified dilutions of each fraction were made, however, and employed throughout the series of tests. This served to standardize the amount of reacting substance present in each antigenic solution. Fraction 1 "undiluted" represents a 1:20 concentration of histoplasmin; fractions 2, 3, and 4 "undiluted," approximately 1:50 concentrations of histoplasmin. Some of each fraction was known to have been lost during the purification process.

Histoplasmin, blastomycin, and fraction 1 gave positive precipitin tests with serum obtained from both groups of rabbits. These antigens were therefore nonspecific. Fractions 2 and 3 appeared more specific for antibodies stimulated by *H. capsulatum*, since in low dilution they reacted with serum obtained from all the *H. capsulatum*-inoculated rabbits but with serum obtained from only 1 of the *B. dermatitidis*-inoculated rabbits. Fraction 4 reacted in low dilution with 4 out of 7 sera from the *H. capsulatum*-inoculated rabbits but failed to react with any of the sera obtained from those inoculated with *B. dermatitidis*.

It is of interest to note that the presence of antibodies in fractions 2, 3, and 4 was of short duration, being demonstrable for a period of not more than 3 weeks. Antibodies to histoplasmin and blastomycin, on the other hand, could still be demonstrated in some of the rabbits 9 weeks after injection.

Skin tests were performed on 5 rabbits injected with *H. capsulatum* and the 3 rabbits injected with *B. dermatitidis* which were still living 9 weeks after inoculation. Fractions 1 and 2 were not specific for *H. capsulatum* when used in 1:100 or a 1:1,000 dilution, as they gave positive skin reactions in both groups of rabbits. Fraction 3 gave negative results in all the rabbits when used in a 1:100 dilution.

Fraction 4 in a 1:10 dilution gave positive reactions in 4 out of 5 of the rabbits injected with *H. capsulatum* and negative results in the remaining 2 animals injected with *B. dermatitidis*. This would indicate that fraction 4 is the most specific of the fractions, since these 2 rabbits had reacted positively to one or more dilutions of fractions 1 and 2.

The 3 normal rabbits reacted negatively to all the antigens when injected intradermally.

The results of the studies presented above indicate that precipitin tests may be of value as an aid in the diagnosis of histoplasmosis and blastomycosis. Fractions obtained from the broth filtrate of the mycelial phase of *H. capsulatum* give more promise of being specific for *H. capsulatum* infections than the broth filtrate itself.

A more detailed account of the work presented here will be published at a later date.

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The Occurrence of Temperatures Unusual to American Lakes[1]

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Lake waters are known to exhibit two major temperature characteristics of primary ecological significance. One of them is the existence of seasonal changes characterized by surface-bottom mixing in the spring and fall seasons, with intervening periods of relatively stable thermal conditions. The second widely occurring phenomenon, known since the work of Simony about 1850, is the rapid decrease in temperature throughout an intermediate water layer, termed the *thermocline* by Birge in 1897. Above the thermocline is a layer called the *epilimnion* that usually extends to a depth of about 12–24′ or more below the surface, while below it is the *hypolimnion* layer in which the water, under normal conditions, is disturbed only during the spring and fall seasons by the surface-bottom mixing of the waters of the lake.

The morphological, metabolic, and physical-chemical properties of different lakes are directly related to variation with respect to these two dominant characteristics. This paper describes briefly three departures from the temperature conditions that usually exist in lakes. They are as follows: (1) a measurable increase in temperature from the top to the bottom of the hypolimnion; (2) the permanent stagnation of the hypolimnion throughout the year; and (3) the prolonged existence of the upper limit of the epilimnion to within about a foot or two of the surface.

Observations were made at Sodon Lake, Oakland County, Michigan (Bloomfield Township, Sect. 20; lat. 42° 19′, long. 83° 17′) during the period May 1947–May 1948. The first record of a temperature increase in the hypolimnion in this lake was made on May 22, 1947, by Stanley A. Cain and the senior writer. Since that time an intensive study has been made of the dominant physical and chemical properties of this lake in an effort to associate the unusual thermal properties of the water layers with related phenomena.

Sodon Lake is a small, ice-block lake from 50′ to 60′ in maximum depth and 5.7 acres in area at the surface, 3.2 acres within the 20′ depth contour, and 1 acre within the 40′ isobath. The volume development of the lake is 1.21, indicating that the basin closely approximates a cone.

Considerable protection from wind action is afforded by the surrounding wooded hillsides and, to a lesser ex-

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