THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 45, No. 5

Printed in U.S.A.

THE ANTIFUNGAL ACTIVITY OF PHYSIOLOGIC SALINE IN SERUM*

NINA DABROWA, M.A., JOSEPH W. LANDAU, M.D. AND VICTOR D. NEWCOMER, M.D.

Physiologic saline (0.85% sodium chloride) is widely used for the suspension of microorganisms and for the dilution of sera in numerous microbiological and serological procedures. It is usually considered innocuous and has the advantage over distilled water of maintaining isotonicity of serum and preventing hemolysis of any added erythrocytes.

Certain observations, however, suggest that physiologic saline may in some instances exert an undesirable effect. The addition of sodium chloride to constitute 0.5% or more of a medium produced an apparent inhibition of growth of *Aerobacter aerogenes* compared with its growth in the absence of this salt (1). The titer of antinuclear factor was found to be consistently lower in positive sera after dilution with physiologic saline than after dilution with a phosphate buffer of lower ionic strength (2).

Little information is available concerning any deleterious activity of physiologic saline on the growth of fungi pathogenic to man. Some nonpathogenic fungi isolated from oceans and estuaries actually grow more extensively on media prepared with a solution containing approximately 3% sodium chloride than on media prepared with distilled water (3). The growth of *Coccidioides immitis, Hormodendrum (Phialophora) compactum, Sporotrichum schenckii* and *Trichophyton mentagrophytes* as estimated by measuring the colony diameter has been demonstrated to be less on Sabouraud media prepared with physiologic saline compared with that on media prepared with distilled water (4). A toxic effect of physiologic saline on Candida organisms has previously been observed but the details were not reported (5). *Candida albicans* has been found to remain viable after incubation at room temperature for at least six weeks in distilled water and in solutions containing as much as eight times the physiologic concentration of sodium chloride (4).

Investigations in this laboratory and others have attempted to define the factors in human serum that influence the growth of *C. albicans*. The yeast cells of *C. albicans* readily form filaments or "germ tubes" within three hours when incubated at 37° C in serum (6, 7). The property of serum responsible for this rapid germ tube formation has been characterized as heat-stable, not removed by dialysis, unrelated to Candida agglutinins and precipitins, and absorbed by viable and non-viable homologous cells (7). The adverse effects of human serum on the growth of *C. albicans* have been observed in other *in vitro* systems utilizing turbidimetry and quantitative plating technics (8, 9). The relationship of this latter antifungal property of serum to that stimulating rapid germ tube formation has not been satisfactorily clarified and current evidence suggests that both are distinct from classical antibody (7–9). The saturation with iron of the serum protein, transferrin, has recently been demonstrated to result in an increase in both the percentage of germ tubes and the number of colonies of *C. albicans* isolated from such sera (10). A small increase in the percentage of germ tubes and the number of colonies was observed to follow the dilution with distilled water of serum with either unsaturated or saturated transferrin to one-third of its original volume (10).

The purpose of this report is to present the results of studies evaluating the role of physiologic saline and distilled water as diluents of serum on the formation of germ tubes and production of colonies by *C. albicans*.

This study was supported in part by USPHS Grants A1-01478-08, 5 T1 A1 52-05, A1-06048-01, and the Dermatologic Research Foundation of California, Inc.

Presented at the Twenty-sixth Annual Meeting of The Society for Investigative Dermatology, Inc., New York, N.Y., June 21, 1965.

* From the Department of Medicine, Division of Dermatology, University of California, The Center for the Health Sciences, Los Angeles, California 90024 and Medical Service, Veterans Administration Center, General Medical and Surgical Hospital, Los Angeles, California 90025.

MATERIALS AND METHODS

Strains of C. albicans

A strain of *C. albicans* described previously (7, 10) was used. In several experiments three additional strains were used.

Media

Sera obtained from fasting healthy humans were separated from the clots by centrifugation, stored at 4° C, and used within several days. The equipment utilized in this study was not specially processed to remove iron and the transferrin of the sera, as determined on several occasions, was considered to be saturated with iron. No adjustments for pH were made since the final pH of each test medium containing serum was between 7.0 to 7.4.

Tissue culture medium 199, full strength (Hyland Laboratories), was also used.

Diluents

These included distilled water, physiologic saline containing 0.85% sodium chloride in distilled water, 0.2% dextrose in physiologic saline, 0.2% dextrose in distilled water, and sodium chloride in distilled water in concentrations of 0.425%, 1.7% and 3.4%. Solutions in distilled water of Allied Chemical "reagent quality" potassium chloride (KCl), sodium phosphate monobasic (NaH_2PO_4), potassium phosphate monobasic (KH_2PO_4), sodium sulfate (Na_2SO_4), potassium sulfate (K_2SO_4), sodium nitrate ($NaNO_3$) and potassium nitrate (KNO_3) were prepared to contain the same concentration of cations (Na or K ions) as in physiologic saline. Physiologic saline was also prepared, in one instance with sodium chloride, Mallinckrodt Chemical, "analytical reagent." The sodium chloride and dextrose used to prepare these solutions were obtained from several different sealed containers. All solutions were sterilized prior to use by autoclaving at 121° C for 15 minutes at 15 lb/sq in. of pressure.

Determination of Percentages of Germ Tubes and Budding Cells

These procedures were performed as described previously (7, 10) with 0.5 ml of test medium and 0.05 ml of an inoculum of *C. albicans* containing approximately 10^7 cells per ml.

Determination of Number of Colonies

The quantitative plating technique was performed as described previously (7, 10) with 0.9 ml of test medium and 0.1 ml of an inoculum of *C. albicans* containing 2×10^3 cells per ml. The period of incubation was 72 hours except where otherwise stated.

In one instance similar quantitative colony counts were performed with 0.1 ml of the larger inoculum employed in the germ tube determinations.

Studies of both germ tubes and colonies with any single test medium were performed in duplicate and the average recorded as the result. All experiments were repeated on several occasions with freshly prepared diluents and inocula for the purpose of controlling any laboratory variables.

Statistics

The t test and sign test were employed and a probability of occurrence of $p < .05$ was considered statistically significant (11).

EXPERIMENTS AND RESULTS

Experiment 1

The formation of germ tubes and budding cells in serum diluted with physiologic saline or distilled water.—Sera were examined before and after dilution to 75%, 50%, 25% and 5%. A progressive decrease in the percentage of germ tubes and an increase in the percentage of budding cells followed the dilution of each serum with increasing proportions of physiologic saline. In all instances at each of the dilutions a higher percentage of germ tubes and a lower percentage of budding cells developed in a serum diluted with distilled water compared with those in the same serum similarly diluted with physiologic saline. These findings are statistically significant and are presented in Table I. The mean results are recorded in Figure 1. A statistically significant decrease in the mean percentage of germ tubes in sera diluted with distilled water compared with that in undiluted sera was not attained until the serum concentration was reduced to 5%. A statistically significant change in the mean percentage of budding cells compared with that in undiluted sera was found only in the sera which were diluted to 5% with physiologic saline.

Experiment 2

The development of colonies from serum diluted with physiologic saline or distilled water.—Sera were examined before and after dilution to 50% and to 5%. The results are recorded in Table II and Figure 2. No statistically significant differences were found between the numbers of colonies developing from distilled water, physiologic saline, undiluted sera, sera diluted to 50% and to 5% with physiologic saline, and sera diluted to 50% with distilled water. In all instances a statistically

TABLE I

The formation of germ tubes and budding cells by C. albicans *in sera diluted with physiologic saline, distilled water, and 0.2% dextrose in physiologic saline*

	Percentage of Germ Tubes											Percentage of Budding Cells					
Percentage of Serum...	100%	75%		50%			25%		5%			100%	50%		5%		
Diluent.....		Saline	Water	Saline	Water	Dextrose in saline	Saline	Water	Saline	Water	Dextrose in saline		Saline	Water	Saline	Water	Dextrose in saline
Serum #1	78	60	70	27	75	—	17	80	3	27	—	—	—	—	—	—	—
2	51	35	60	29	60	—	27	66	7	21	—	—	—	—	—	—	—
3	70	42	59	37	67	—	15	70	3	33	—	—	—	—	—	—	—
4	70	44	60	35	78	69	12	76	3	38	14	8	23	12	65	44	78
5	70	—	—	45	67	85	—	—	17	52	47	8	15	7	44	17	35
6	62	—	—	41	75	71	—	—	18	53	55	16	25	7	42	21	22
7	78	—	—	55	86	70	—	—	10	57	28	16	20	8	40	19	55
8	63	—	—	43	69	76	—	—	14	56	32	15	32	9	53	21	62
9	44	—	—	28	55	71	—	—	10	46	45	41	52	27	66	42	—
10	78	—	—	67	84	—	—	—	55	91	—	7	11	10	20	2	—
11	68	—	—	58	75	—	—	—	7	50	—	8	13	10	59	23	—
12	78	—	—	60	90	—	52	85	45	89	—	12	11	4	26	3	—
13	76	—	—	36	87	47	—	—	—	—	—	—	—	—	—	—	—
14	67	—	—	30	87	46	—	—	—	—	—	—	—	—	—	—	—
15	73	—	—	4	96	61	—	—	—	—	—	—	—	—	—	—	—
16	88	—	—	26	97	82	—	—	—	—	—	—	—	—	—	—	—
17	77	—	—	12	90	58	—	—	—	—	—	—	—	—	—	—	—
18	84	—	—	25	90	81	—	—	—	—	—	—	—	—	—	—	—
19	90	—	—	23	85	86	—	—	—	—	—	—	—	—	—	—	—

significant increase in the number of colonies was obtained from the sera diluted to 5% with distilled water when compared with the numbers of colonies from the other media.

The importance of the period of incubation was studied by determining the numbers of colonies developing from a single serum after one to seven days. The results are presented in Figure 3. The number of colonies from undiluted serum remained essentially unchanged. An increased number of colonies developed from sera diluted to 5% with physiologic saline or with distilled water as the incubation period was prolonged. A greater number of colonies was consistently obtained from the serum diluted with distilled water. Decreased numbers of colonies were isolated from physiologic saline and from distilled water after prolonged incubation. The numbers of colonies from sera diluted to 50% with either physiologic saline or distilled water did not differ. Both increased from 10^2 per ml after one day to 10^3 per ml after seven days.

When the larger inoculum employed for the germ tube procedure was used, no differences were observed between the numbers of colonies from physiologic saline, distilled water, undiluted serum, serum diluted to 50% and to 5% with distilled water or with physiologic saline following incubation for one to seven days.

Experiment 3

The development of germ tubes in, and colonies from, serum diluted with 0.2% dextrose in physiologic saline.—The percentage of germ tubes in sera diluted to 50% and to 5% and the number of colonies from sera diluted to 5% were determined. The results are presented in Tables I and II and Figure 4. The mean per-

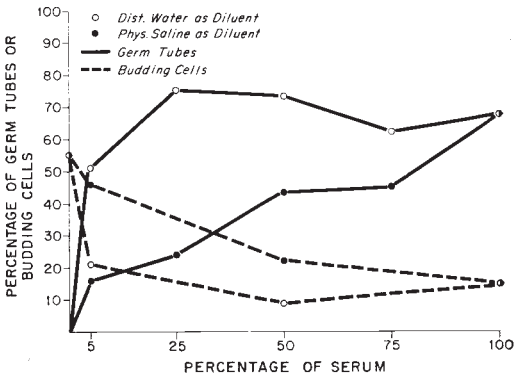

FIG. 1. The mean percentages of germ tubes and budding cells of *C. albicans* in serum diluted with physiologic saline or distilled water.

TABLE II

The number of colonies of C. albicans *per ml after 72 hours of incubation in sera diluted with physiologic saline, distilled water, or 0.2% dextrose in physiologic saline*

Diluent	Percentage of Serum						
	100%	50%			5%		
		Saline	Water	Dextrose in saline	Saline	Water	Dextrose in saline
Serum #1	5×10^2	8×10^3	3×10^3	3×10^3	6×10^2	1×10^5	6×10^5
2	2×10^3	3×10^3	2×10^3	5×10^3	2×10^3	7×10^4	1×10^5
3	8×10^3	5×10^3	5×10^4	2×10^4	7×10^3	7×10^5	1×10^5
4	4×10^2	8×10^3	3×10^3	—	1×10^3	1×10^5	—
5	4×10^3	8×10^3	3×10^3	—	1×10^3	1×10^5	—
6	5×10^3	9×10^3	2×10^4	—	3×10^2	3×10^5	—
7	1×10^2	2×10^2	3×10^3	—	8×10^2	1×10^4	—
8	5×10^2	1×10^3	2×10^3	1×10^3	—	—	—
9	3×10^2	2×10^2	6×10^3	1×10^2	—	—	—
10	1×10^2	1×10^2	1×10^3	1×10^2	—	—	—
11	5×10^2	2×10^3	1×10^3	3×10^3	—	—	—
12	1×10^2	—	—	—	2×10^2	3×10^4	3×10^4
13	1×10^3	—	—	—	1×10^2	3×10^3	1×10^4
14	1×10^3	—	—	—	1×10^2	2×10^3	3×10^5
15	1×10^3	—	—	—	4×10^3	5×10^4	1×10^4

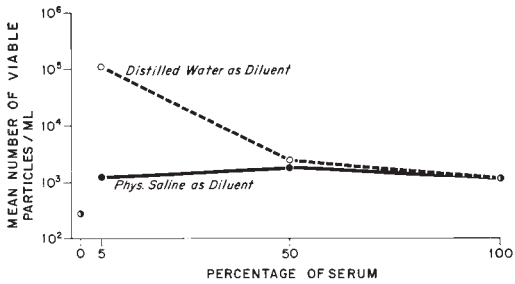

FIG. 2. The mean numbers of colonies of *C. albicans* from serum diluted with physiologic saline or distilled water.

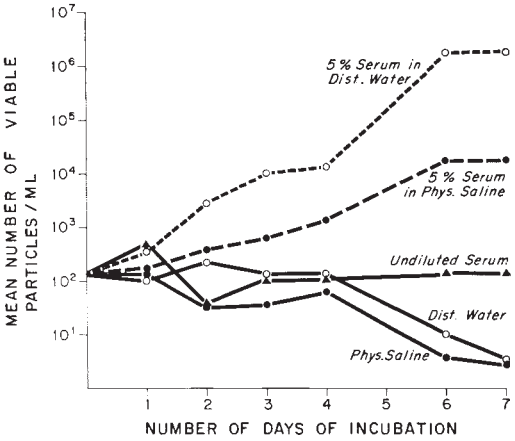

FIG. 3. The numbers of colonies of *C. albicans* developing after incubation for one to seven days in undiluted serum, physiologic saline, distilled water, and serum diluted with physiologic saline or distilled water.

centage of germ tubes and number of colonies from the sera diluted with dextrose in saline are significantly greater than those from sera diluted with physiologic saline but are not significantly different from those sera diluted with distilled water.

A single serum was diluted to 75%, 50%, 25% and 5% with distilled water, physiologic saline, 0.2% dextrose in saline, and 0.2% dextrose in distilled water. The percentage of germ tubes following dilution with the two latter solutions paralleled that following dilution with distilled water. The results are presented in Figure 5.

No differences were found between the numbers of colonies developing from seven sera diluted to 50% with dextrose in saline, physiologic saline or distilled water.

Experiment 4

The development of germ tubes in, and colonies from, serum diluted with solutions containing increasing concentrations of sodium chloride.—The percentage of germ tubes was determined in a single serum diluted with distilled water and solutions containing sodium chloride dissolved in distilled water in concentrations of 0.425%, 0.85%, 1.7% and 3.4%. A progressive decrease in the percentage of germ tubes followed dilution with increasing proportions of each solution and also followed dilution with the same proportion but increas-

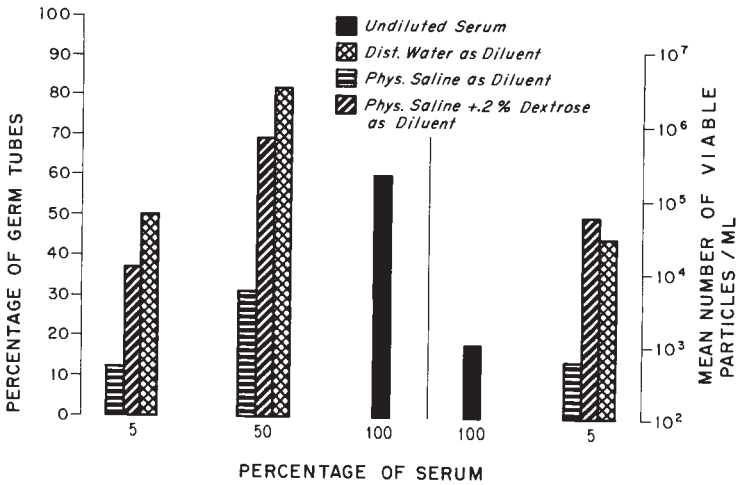

FIG. 4. The mean percentages of germ tubes and numbers of colonies of *C. albicans* from serum diluted with physiologic saline, 0.2% dextrose in physiologic saline, or distilled water.

ing concentrations of sodium chloride. The results are presented in Figure 5.

The development of colonies was also determined after dilution of the serum to 5% with these solutions. The number of colonies from serum diluted with the 0.425% sodium chloride solution was the same as that from serum diluted with distilled water. The numbers of colonies from the serum diluted with the 1.7% and the 3.4% solutions were the same as those from the serum diluted with physiologic saline.

Experiment 5

The development of germ tubes in, and colonies from, serum diluted with solutions containing various ions.—The percentage of germ tubes in and number of colonies from sera diluted to 50% and to 5% were determined with solutions containing the same concentration of cations (.15M) as in physiologic saline. The mean results of two separate determinations are presented in Figures 6 and 7. The percentage of germ tubes was less in the sera diluted with the sodium salts than in sera diluted with the potassium salt of each compound at both 50% and 5% dilutions. The number of colonies from sera diluted to 50% and to 5% with the various salt solutions showed little change from serum diluted with distilled water with the exception of a small decrease following dilution with the sodium sulfate solution and a large increase following dilution with either phosphate salt solution. The numbers of colonies in the sera diluted to 5% were less than in sera diluted to 50% with the phosphate salt solutions in contrast to the increase in number of colonies derived from serum diluted with distilled water.

The various diluting solutions without added serum were also examined. No germ tubes developed and the percentage of budding cells ranged from 60 to 70 in each of the solutions. After 72 hours of incubation, the number of colonies per ml ranged between 1×10^2 to

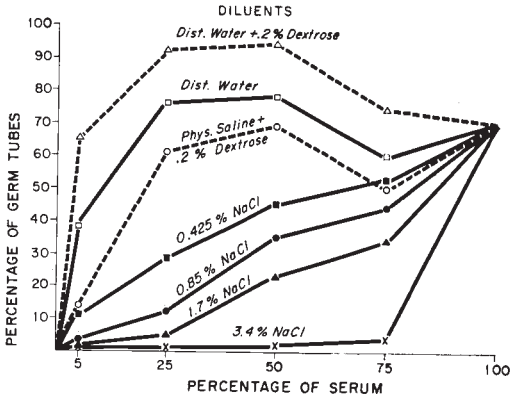

FIG. 5. The percentage of germ tubes of *C. albicans* in a serum diluted with distilled water or solutions of dextrose and various concentrations of sodium chloride.

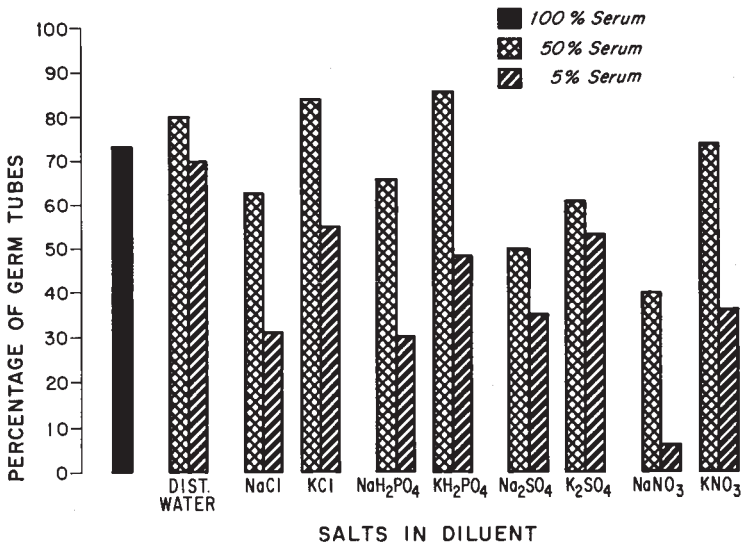

FIG. 6. The percentages of germ tubes of *C. albicans* in sera diluted with solutions of various salts containing the same concentration of cations as in physiologic saline.

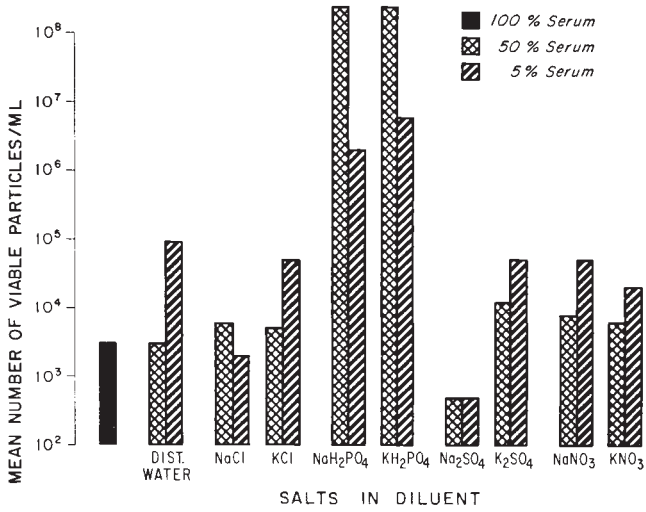

FIG. 7. The numbers of colonies of *C. albicans* from sera diluted with solutions of various salts containing the same concentrations of cations as in physiologic saline.

1×10^8 with the exception that 5×10^4 colonies developed from the potassium phosphate monobasic solution.

Experiment 6

The development of germ tubes and budding cells in, and colonies from, tissue culture medium 199 diluted with various solutions.—Different lots of the medium were examined before and after dilution to 50% and to 5% with physiologic saline and distilled water. The results are presented in Table III and Figure 8. Dilution with distilled water did not statistically significantly alter the mean percentage of germ tubes or budding cells. Dilution with physiologic saline produced a progressive decrease in the percentage of germ tubes and an increase in the percentage of budding cells. The differences in the mean results in the media diluted to 5% with physiologic saline compared with those in the undiluted media are statistically significant. In all instances at both 50% and 5% dilutions, a statistically significant higher percentage of germ tubes and lower percentage of budding cells developed in the medium diluted with distilled water compared with the results in the medium comparably diluted with physiologic saline. Media were also diluted on five occasions to 50% and to 5% with 0.2% dextrose in physiologic saline. The percentages of germ tubes and budding cells in these dilutions were not statistically significantly different from the results following dilution with physiologic saline.

Media were also diluted on four occasions to 50% and to 5% with concentrations of potassium chloride equivalent to physiologic saline. In each instance the percentage of germ tubes in the medium diluted with the KCl solution was higher than that in the medium similarly diluted with physiologic saline.

The number of colonies developing in undiluted medium ranged from 10^6 to 10^8 per ml and no differences were found after dilution to 50% or to 5% with either physiologic saline or distilled water.

Similar findings in this experiment and experiments 1, 2 and 3 were demonstrated with the three other strains of *C. albicans* and with diluents prepared from different sources of sodium chloride or dextrose.

DISCUSSION

This study demonstrates that the addition of physiologic saline to serum exerts a deleterious effect on both the production of germ tubes and the development of colonies by *C. albicans* incubated in the serum. The decrease in the percentage of germ tubes in serum diluted with increasing proportions of physiologic saline is principally a manifestation of this antifungal activity. The finding cannot be attributed to a reduction in available essential nutrients since comparable decreases do not occur in serum

TABLE III

The formation of germ tubes and budding cells of C. albicans *in medium 199 diluted with physiologic saline or distilled water*

Percentage of Medium 199	Percentage of Germ Tubes					Percentage of Budding Cells				
	100%	50%		5%		100%	50%		5%	
Diluent		Saline	Water	Saline	Water		Saline	Water	Saline	Water
Medium #1	81	75	90	51	74	7	18	7	38	18
2	65	35	84	13	51	29	54	8	79	39
3	86	57	91	18	78	9	27	5	60	18
4	62	40	66	13	43	34	42	29	77	51
5	48	53	90	50	89	38	18	4	27	4
6	62	34	62	21	89	25	52	27	25	6

similarly diluted with distilled water. A previous report by Mackenzie (6) that the percentage of germ tubes decreases with decreasing concentration of serum can not be evaluated since the diluent used was not stated. The inhibitory effect of physiologic saline on the development of colonies is relative rather than absolute, and was demonstrated by comparing the number of colonies from serum diluted to 5% with physiologic saline with the increased number of colonies from serum similarly diluted with distilled water.

The increased percentage of germ tubes and number of colonies of *C. albicans* from serum diluted with a solution containing dextrose in saline compared with that from serum diluted with saline is analogous to the ability of some fungi isolated from estuarine sediments to adapt readily *in vitro*, when ample nutrients are provided, to salinity levels far in excess of that to which they otherwise can tolerate (3). The formation of germ tubes (7) in undiluted serum has been found to be unaffected by the addition of dextrose in amounts equivalent to 10 mg per ml, but the growth (12) of *C. albicans* as determined by turbidimetry after 24 hours was enhanced by the addition of dextrose to serum in amounts equivalent to 1.5 mg per ml or more. Progressive loss of inhibitory activity for *C. albicans* of mouse ascites fluid also results from adding increased concentrations of dextrose (13).

No definitive decisions can be made concerning the relative importance of the sodium ion compared with the chloride ion in determining the development in serum of either germ tubes or colonies. Salts of the sodium ion appeared

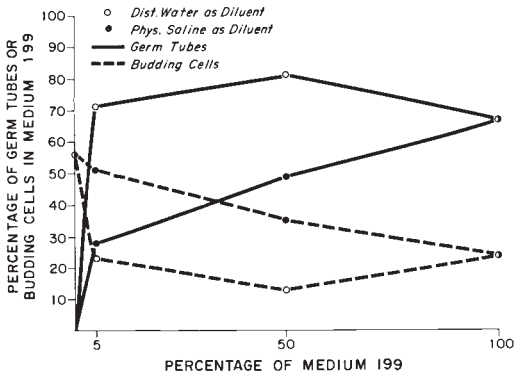

FIG. 8. The mean percentages of germ tubes and budding cells of *C. albicans* in tissue culture medium 199 diluted with physiologic saline or distilled water.

to inhibit the formation of germ tubes to a greater extent than salts of the potassium ion but this observation can not be extended to conclude that the sodium ion is responsible for the inhibitory activity in physiologic saline. The chloride ion and other anions may, conceivably, all be inhibitory and this may have been neutralized in the presence of a possible germ tube promoting effect of the potassium ion. Studies with additional salts will resolve this point. Analysis of the effect of these ions on the subsequent development of colonies is more complex and conclusions based on the available data would be tenuous. The osmotic pressure in physiologic saline does not appear to be an important factor since both the percentage of germ tubes and the number of colonies from serum diluted with the solution of potassium chloride parallel those from serum diluted with distilled water. The incorporation of either 10%

sodium chloride or 10% potassium chloride into solid culture media has been found to result in the enhancement of filament formation with some inhibition of total growth (14). The possibility that the inhibitory activity of physiologic saline is caused by an impurity in the sodium chloride preparation is unlikely but this cannot be entirely excluded. The reagents, however, contained not over a total of 0.5% impurities.

An unresolved question has been whether the rapid formation of germ tubes in serum represents enhancement or inhibition of the number of colonies of *C. albicans,* subsequently developing from the serum (7). The increases in both the percentage of germ tubes and the number of colonies observed after saturation of transferrin with iron implied a possible parallel relationship (10). This correlation, however, could not be extended to other media since in studies utilizing Sabouraud broth a high number of colonies and low percentage of germ tubes developed (10). Increases in both the percentage of germ tubes and the number of colonies followed the addition of dextrose to serum diluted to 5% with physiologic saline. Increases in the number of colonies and decreases in the percentage of germ tubes, however, occurred in serum diluted to 5% with distilled water or with solutions of the phosphate salts compared with the findings in undiluted serum. The relationship in serum between the percentage of germ tubes and the number of colonies appears to be dependent on a variety of factors and no generalizations can be stated convincingly at this time.

The decrease in percentage of germ tubes by *C. albicans* in tissue culture medium 199 diluted with physiologic saline indicates that the presence of serum protein is not essential for inhibitory activity of physiologic saline. The inability of dextrose to reverse this inhibitory activity in this instance and the failure to demonstrate any difference between dilution with distilled water and with physiologic saline on the development of colonies, however, supports the contention (10) that the mechanism by which germ tube formation occurs in serum and medium 199 may not be identical.

The findings in these experiments provide additional support for the concept that normal human serum possesses an inhibitory activity against *C. albicans.* (7–10) The increase in the number of colonies from serum diluted to 5% with distilled water after 72 hours incubation and from serum similarly diluted with physiologic saline after a longer incubation period compared with that from undiluted serum implies that a reduction in this inhibitory activity can be accomplished by dilution. At the 5% serum concentration the nutrients are quite capable of maintaining satisfactory growth of *C. albicans.* The inhibitory property is still present in serum diluted to only 50% with distilled water but can be overcome by the addition of phosphate salts. Phosphate salts have been found to reverse the tuberculostatic activity (15) of normal human serum and are also known to influence the growth of *C. albicans* (11, 16). Whether or not the normal concentrations of sodium and chloride ions contribute to the antifungal activity in serum has not been determined but is currently being investigated. Sodium chloride presumably is not the sole factor since inhibitory activity has previously been demonstrated in the unsaturated transferrin of serum (10). Moreover, no proof is yet available to indicate that any *in vitro* antifungal property of serum has an importance in the normal defense mechanisms of man.

SUMMARY

Physiologic saline exerts an adverse effect on the development of both germ tubes and colonies of *C. albicans.* The decrease in the percentage of germ tubes which occurs in human serum and in tissue culture medium 199 diluted with increasing proportions of physiologic saline does not occur when these media are similarly diluted with distilled water. The number of colonies, after 72 hours of incubation, from human serum diluted to 5% with physiologic saline is the same as that from undiluted serum but is approximately a hundredfold less than that from serum diluted to 5% with distilled water. The inhibitory property of physiologic saline is not demonstrable when 0.2% dextrose is present in the physiologic saline used to dilute the serum. The percentage of germ tubes is less in sera diluted with the sodium salt than in sera diluted with the potassium salt of chloride, sulfate, monobasic phosphate or nitrate at both 50% and 5% dilutions.

The results of this study also provide additional evidence for the presence of antifungal activity in normal human serum and for the

lack of any constant relationship beween the percentage of germ tubes and the number of colonies.

REFERENCES

1. Naylor, P. G. D.: Inhibition of growth of *Aerobacter aerogenes* by sodium chloride. Nature (London), *205:* 420, 1965.
2. Laffin, R. J., Bardawil, W. A., Pachas, W. N. and McCarthy, J. S.: Immunofluorescent studies on the occurrence of antinuclear factor in normal human serum. Amer. J. Path., *45:* 465, 1964.
3. Borut, S. Y. and Johnson, T. W., Jr.: Some biological observations on fungi in estaurine sediments. Mycologia, *54:* 181, 1962.
4. Dzawachiszwili, N., Landau, J. W., Newcomer, V. D. and Plunkett, O. A.: The effect of sea water and sodium chloride on the growth of fungi pathogenic to man. J. Invest. Derm., *43:* 103, 1964.
5. Andriole, V. T. and Hasenclever, H. F.: Factors influencing experimental candidiasis. I. alloxan diabetes. Yale J. Biol. Med., *35:* 96, 1962.
6. Mackenzie, D. W. R.: Serum tube identification of *Candida albicans*. J. Clin. Path., *15:* 563, 1962.
7. Landau, J. W., Dabrowa, N. and Newcomer, V. D.: The rapid formation in serum of filaments by *Candida albicans*. J. Invest. Derm., *44:* 171, 1965.
8. Roth, F. J., Jr. and Goldstein, M. I.: Inhibition of growth of pathogenic yeasts by human serum. J. Invest. Derm., *36:* 383, 1961.
9. Louria, D. B. and Brayton, R. G.: A substance in blood lethal for *Candida albicans*. Nature (London), *201:* 309, 1964.
10. Landau, J. W., Dabrowa, N., Newcomer, V. D. and Rowe, J. R.: The relationship of serum transferrin and iron to the rapid formation of germ tubes by *Candida albicans*. J. Invest. Derm., *43:* 473, 1964.
11. Dixon, W. J. and Massey, F. J., Jr.: Introduction to Statistical Analysis. New York, McGraw-Hill, 1951.
12. Johnson, S. A. M.: *Candida* (*monilia*) *albicans*. Arch. Derm. (Chicago), *70:* 49, 1954.
13. Summers, D. F. and Hasenclever, H. F.: In vitro inhibition of yeast growth by mouse ascites fluid and serum. J. Bact., *87:* 1, 1964.
14. McClary, D. O.: Factors affecting the morphology of *Candida albicans*. Ann. Missouri Botan. Garden, *39:* 137, 1952.
15. Kochan, I., Patton, C. and Ishak, K.: Tuberculostatic activity of normal human sera. J. Immun., *90:* 711, 1963.
16. Widra, A.: Phosphate directed Y-M variation in *Candida albicans*. Mycopathologia, *23:* 197, 1964.

DISCUSSION

DR. MARSDEN S. BLOIS JR., Atherton, California: There is a semantic distinction that might be made here. When we talk about "physiologic saline" we are talking about what is physiologic for humans. As far as *Candida albicans* is determined, this may be unphysiologic saline. Instead of trying solutions of other salts, have you carried out the same experiments using different molarities of saline, for example half or tenth normal saline?

DR. JOSEPH W. LANDAU (in closing): The point we are stressing is that physiologic saline may be "physiologic" for human blood and tissues but may not be "physiologic" for *Candida albicans*. An experiment with diluents containing different concentrations of sodium chloride was performed and is included in the complete manuscript.